THE UNIVERSITY OF ALBERTA

STAPHYLOCOCCAL MASTITIS IN ALBERTA DAIRY HERDS

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES IN
PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE
DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF BACTERIOLOGY

by

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APRIL 9th, 1960

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in

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled

Staphylococcal Mastitis in Alberta
Dairy Herds

submitted by Heinz Joseph Kraus
in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Staphylococci were found to be of increasing importance as agents of bovine mastitis in Alberta dairy herds. Statistics from the files of the Provincial Dairy Laboratory, Edmonton, showed that the relative importance of staphylococci in the etiology of bovine mastitis had increased rapidly. In the Province of Alberta these organisms have now gained an importance equal to that of streptococci.

The final diagnosis of staphylococcal mastitis is established in the laboratory. Microscopic smear examination was found to be of the greatest diagnostic value. An abnormally high leucocyte count associated with a large number of staphylococci was considered suggestive of staphylococcal mastitis. The diagnosis was held to be confirmed if many of these staphylococci were phagocytized.

Observations on eighty strains of staphylococci isolated as agents of acute mastitis included colonial morphology, production of hemolysins, fermentation of mannite, production of coagulase, phage type, and sensitivity to various antibiotics. Colonial morphology and pigment formation were found to be subject to gross variation and cannot be accepted as reliable criteria for the classification of staphylococci. Fermentation of mannite was observed in seventy-seven of the eighty strains. Most mastitis staphylococci seemed to be mannitol fermenters, but numerous staphylococcal strains isolated from normal udders were also able to ferment mannitol. Therefore, this character was not considered a reliable indication of pathogenicity. Seventy-three strains produced hemolysin, seven strains in this series were non-hemolytic; strains of this sort are not uncommon as agents of mastitis. Some evidence was found to suggest that the ability of a strain to produce hemolysin may be suppressed by some factor present in

mastitic quarters, and may be potentiated by a diffusable product of alpha-streptococci. Of the eighty strains, eleven did not produce coagulase. Although the ability to produce coagulase may increase the pathogenic potential of a strain, pathogenicity is certainly not dependent upon this character. Coagulase negative strains can cause mastitis. The ability to produce coagulase, however, was found to be a fairly stable character and therefore the division of staphylococci into coagulase positive and coagulase negative strains is acceptable.

The strains were typed by the Rippon and Williams phage system. Types 42 d and 81 were of the highest frequency in mastitis. Observations tended to confirm the belief that the pattern of phage susceptibility is a persistant and dependable character of the staphylococcus, in vivo as well as in vitro. Types 42 d and 81 were found to include both coagulase positive and coagulase negative strains. In some instances at least the phage type seemed to be more significant than coagulase production as an indicator of pathogenicity.

It was observed that many staphylococci isolated as agents of mastitis were resistant to one or more antibiotics. Evidence suggested that this phenomenon was due to the emergence of resistant mutants. Irregular use and regular abuse of antibiotics in treatment initiated and carried out by the herd management were suggested reasons for this development.

A mastitis control program was designed and carried out in two Alberta dairy herds in which major outbreaks of staphylococcal mastitis had occurred. It was shown that the outbreaks in herd A originated mainly from negligence in herd management while in herd B the continuous misuse of antibiotics was considered a principal

cause. The main steps in this program were: Instruction of personnel including an explanation of the character of the disease and its agent; elimination of predisposing factors; prevention of transmission; selection of treatment based on laboratory findings; rules for establishment of permanent herd hygiene. Excellent results with some promise of permanence were obtained. Those results suggest that the control of bovine mastitis is mainly a matter of good and effective herd management. Treatment with antibiotics should be considered a last resort and should be administered only by competent persons.

Possible reasons for the increased importance of staphylococci were considered. The ubiquity and the high degree of variability of staphylococci were suggested as principal factors. In these respects staphylococci were more effective agents of mastitis than *Streptococcus agalactiae*.

Finally, the necessity of revising conventional ideas on the control of bovine mastitis is emphasized. This is particularly necessary on account of the current general use of mechanical milking which results in a heavy stress on the animals and makes them peculiarly liable to infections with organisms such as staphylococci.

Acknowledgements

To Dr. R.D. Stuart, Professor of Bacteriology and Director of the Provincial Laboratory of Public Health, Edmonton, for his constant supervision and guidance;

To Dr. E. Williams for bacteriophage typing of cultures of staphylococci;

To the Department of Agriculture of the Province of Alberta for permitting the use of facilities and materials of the Provincial Dairy Laboratory for this investigation;

My grateful thanks.

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INTRODUCTION

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I. The Importance of the Mastitis Problem in the Dairy Industry

(1) Hoard's "Dairyman", a leading U.S. publication in the dairy field, deals in four successive issues (March - April, 1959) with recent observations on bovine mastitis (1) A general comment on the mastitis problem reads "Of all the subjects we discuss in our columns, mastitis is the most vexatious, stubborn, controversial, complicated, and frustrating." As reasons for this view on the problem, the article mentions:

a.) Mastitis is caused by a variety of microorganisms.

b.) Authorities disagree on the best approach to control.

c.) Little research is being done.

d.) A mechanical device, the milking machine, influences udder health.

e.) Innumerable antibiotics are available for treatment. But there is a question about the value of the sensitivity testing used for the selection of the preferred antibiotic.

f.) Diagnosis is subject to debate. No widely accepted methods are available.

g.) The disease is the most costly in the entire dairy farming industry .

This picture of the mastitis situation reflects very well the confusion of views and the lack of uniformity in control measures. Human art and skill are widely involved in the control of any infectious disease. We deal with living organisms. Only a clear understanding and knowledge of the agents can lead to effective and uniform measures. Whoever becomes scientifically engaged with the mastitis problem must find it difficult to understand why so little is done to broaden the knowledge of etiology and mechanism of the disease and to pass the results of such a research on to the dairyman who is suffering heavy losses by the disease year after year. The United States dairy industry estimates the loss due to mastitis at $225,000,000 every year (2a). The Alberta Department of Agriculture calls a loss of $2,000,000 per annum a conservative estimate (3). Animals suffering from mastitis give less milk. This decrease in production may be perpetuated as mastitis frequently causes an irreparable destruction of part of the milk producing tissues in the udder. Thus the cow's years in the milk line are shortened and more replacements are necessary. Even in a slight infection the percentage of butterfat in the milk is reduced. In more severe cases, the composition of the product may be altered in a way that it cannot be considered as milk anymore. There may be a decrease in:

Fat by two-thirds
non-fat solids by one-half
Casein by one-half
Lactose by four-fifths
Ash by one-third

These facts show that mastitis is a disease which must be controlled in the interest of an industry depending greatly on the health of the dairy livestock. Too many dairymen already have accepted mastitis as an evil which cannot be averted. Such an attitude leads the way to a further spread of the disease and to even greater economical losses.

(2) The present mastitis situation in the Province of Alberta as represented by the records of the Provincial Dairy Laboratory:

A complete assessment of the incidence of mastitis in Alberta dairy herds is very difficult. At present no Government regulation is in existence which would enforce the keeping of herd records showing the state of animal health in the individual dairy herds. It is known that the majority of dairy farmers still try to solve the mastitis problem in their own way, avoiding the consultation of the local veterinarian as long as possible. However, increasing leucocyte counts and mounting antibiotic titers in pooled milk as well as rapidly rising numbers of herd samples submitted to the Provincial Dairy Laboratory for examination on mastitis, suggest that the problem is becoming aggravated and that the figures given in the following paragraph

may not differ significantly from the general situation in the Province.

In the period from November, 1958 to August, 1959 the writer examined 3,282 quarter samples for mastitis. These samples were taken by the local veterinarians and represent thirty-two Alberta dairy herds. In 33 % of the samples, the laboratory diagnosis of mastitis caused by various bacterial agents could be established, 9 % were suspicious of a low grade infection, 58 % were negative. If it is assumed that with very little effort put into an effective control program, the annual infection rate could be reduced to ten to fifteen percent, then ignorance towards the problem seems inexcusable. The possibility of saving the Alberta dairy industry more than $1,000,000. every year should justify and encourage such an effort. If this work contributes to a modest extent towards a clearer picture of the etiology of the disease and a closer knowledge of one of its principal agents, then a very useful purpose is fulfilled.

II. Definition of "Infectious Bovine Mastitis"

(1) Mastitis generally may be defined as an inflammation of the mammary gland or udder, associated with various tissue changes. The etiology may be infectious, chemical, thermal or traumatic. The disease may manifest itself in an acute, subclinical or

subacute, or chronic form. Infectious bovine mastitis has various microorganisms as agents. The invasion of the udder by pathogenic organisms may be spontaneous or the result of chemical, thermal or traumatic damage to the udder tissues. This work is confined to the infectious type of mastitis only.

(2) Acute mastitis involves single quarters or even the entire udder. The reaction may involve not only the parenchyma but also the interstitial tissue and may be accompanied by a systemic disturbance, with a rise in body temperature. This type of mastitis, without regard to etiology, may be referred to as parenchymatous, interstitial or phlegmonous mastitis. When the skin covering the udder or teats also becomes involved, a cellulitis, often associated with lymphangitis, may follow with the production of local or deep abscesses, suppuration and sloughing. Frequently these acute types of infection terminate in a gangrenous form of mastitis, in which the secretory tissue is severely affected. This form of mastitis may be fatal, but even when animals survive, the affected quarters are usually rendered unserviceable.

(3) Subclinical mastitis is identified usually by slight changes in the superficial udder tissues. Slightly swollen quarters, thickened fore-milk may indicate

early, mild, latent, subclinical, or mild catarrhal mastitis. In many instances only delicate biochemical tests show an alteration of the milk. When, in such instances, bacteria, capable of causing mastitis, are cultured from milk of apparently normal quarters it can be assumed that a subclinical mastitis is present. The infection may be temporary or permanent, but it actually exists in the udder until the organisms are eliminated and secretion is normal.

(4) Chronic mastitis may follow the subacute or acute form of the disease. Occasionally, however, chronic mastitis is discovered without any previous clinical symptoms. Chronic mastitis is characterized by a general replacement of the parenchyma or connective tissue. The quarter becomes thickened, firm, nodular, and at times atrophied, and as a result the secretion is abnormal in character and diminished in amount. This is the most common form of mastitis.

(5) A brief review of the physiology of milk secretion may be useful in understanding the damaging effect caused by the agents of the disease, (4a). Milk is secreted in the epithelial cells lining the countless alveoli of the cows udder. Each cell does a complete milk manufacturing job, that is, there are no groups of specialized cells. Each cell secrets the casein, fat, lactose, and other constituents of milk together, and the product of their activity collects in the

lumen of the alveoli. Some of the precursors of milk pass unchanged from the blood stream into the gland. In this case the cell plays a quantitatively selective role. It may concentrate these constituents, and prevent to a variable extent the passage of other constituents from the blood to the milk. This normally precisely balanced mechanism, if exposed to the action of pathogenic organisms, is temporarily or permanently upset in its function. Soon after the agents of mastitis gain entrance to the udder via teat-canal, the products of their metabolism begin to affect the milk producing cells. They gradually lose their normal ability to synthesize the constituents of milk. At the same time the selective permeability of the cell membrane is altered. Blood constituents pass in increasing amounts freely into the milk. In the advanced stages of the infection the composition of the secretory product departs considerably from that of normal milk and approaches the composition of blood serum.

(6) Normal udder flora. For a long time the mode of infection has been a most controversial subject. During 1874 to 1878, Roberts and Lister (6) advanced the theory that milk within the healthy udder is germ-free. This was soon followed by the theory that the udder is inhabited by a "normal flora," consisting mainly of streptococci, micrococci and diphtheroids.

The term "normal udder flora" has since established itself in the minds of many, concerned with the mastitis problem, in a most detrimental way. In fact, it has resulted in the conception of a permanently peaceful parasite-host relationship and in a disregard of the normal flora as a reservoir of potentia pathogens. Even the discovery that the very same organisms are often associated with mastitis did not seem to succeed in making the normal flora suspect. Instead of using the term "normal udder flora" the writer would prefer to speak of a flora usually present in a milk sample which is drawn under aseptic conditions from a quarter free of mastitis. The number of bacterial cells in such a sample should not exceed a limit of 30,000/ml . Leucocytes should be absent except for the few passing incidentally from the bloodstream into the milk. The sample should give a neutral reaction when tested, using brome - cresol - purple as indicator.

111. The Agents of Bovine Mastitis

The more common agents associated with infectious bovine mastitis are streptococci, staphylococci, coliform organisms and C. bovis. It would go beyond the scope of this work to consider in detail the wide variety of individual species of bacteria found as pathogens in mastitis. Streptococci alone are represented by approximately fourteen species. Of special interest, however, in connection with this

work is the relative importance of the different organisms in the etiology of the disease.

A general summary prepared from the reports of seventeen workers (5) in 1944, gives the following order of importance.

Streptococci - 86 %

Staphylococci - 5.4 %

C. pyogenes - 2.7 %

Colongroup - 1. 2 %

Others - 3.7 %

In 1946, Little and Plastridge (4b) attributed 85 % of mastitis outbreaks to streptococci. In a review in 1958 (2) Plastridge reports on a mastitis survey in the States of New York and Connecticut:

Streptococci - 66 %

Staphylococci - 32 %

Others - 2 %

A bulletin on bovine mastitis by the Ontario Veterinary College (7) reports with respect to Staphylococcus pyogenes: "This organism is becoming increasingly more frequent in the herds of this Province."

Table No. 1 shows the relative importance of the principal agents of bovine mastitis in the Province of Alberta during the period 1956 - 1959. The table is based on the results of the bacteriological examination of 14,900 quarter samples as performed at the Provincial Dairy Laboratory, Edmonton.

The relative importance of the principal agents of bovine mastitis in the Province of Alberta

Incidence in Percentage

	1956	1957	1958	1959
Streptococci	62	60	57	46
Staphylococci	30	35	38	45
Streptococci and Staphylocci Combined	5	3	3	6
Others	3	2	2	3
Total of samples submitted	2,910	3,115	4,120	4,755
No. of Positive samples	620	1,140	1,910	2,010

From the material presented comes evidence that a general shift in etiological importance in favor of staphylococci seems to take place. This etiological trend led to the search for possible reasons as discussed in this work.

IV. Staphylococcal Mastitis

(1) Staphylococci in the normal udder. The common occurrence in milk of staphylococci has been reported by many investigators. "Micrococci" that were regarded as inoffensive were found by Gorini 1902 (8) and von Freudenriech (9) to be the most common organisms in freshly drawn milk. Evans (10) obtained micrococci from 58.8 % of the milk samples from udders regarded as normal. About 10 % of these cultures were hemolytic on oxblood agar and were virulent for rabbits. Plastridge et al. (4c) doubt that even non-hemolytic, coagulase negative staphylococci can be considered as belonging to a "normal udder flora." They found that the average leucocyte count of 2,125 milk samples, that were free from staphylococci and other mastitis organisms, was 73,000/cc. On the other hand, the average count for 192 samples that contained non-hemolytic, coagulase negative staphylococci was 240,000/cc. None of the 192 samples was abnormal in appearance and one only reacted suspiciously to the bromothymol blue test.

(2) Staphylococci associated with bovine mastitis: As early as 1889 Lucet (11) reported the finding of gelatine -

liquefying micrococci in the secretion of seven of twenty one animals affected withmastitis. Other early investigators,including Guillebeau (13), Steiger (14), Savage (15), Jones (16), Carpenter (17) and Rolle (18) found that staphylococci were present in a significant proportion of abnormal udder secretions in which streptococci were absent. Little and Plastridge (4d)report that about ten percent of dairy cattle yield milk which contains staphylococci associated with a leucocyte count of 500,000/cc or more. Evidence derived from the results of herd tests in the Province of Alberta suggests that at present a minimum of sixteen percent of dairy cattle produces milk with leucocyte counts over 500,000/cc due to the presence of staphylococci.

(3) Variety of Mastitis produced by Staphylococci: According to Little and Plastridge (4e) the presence of non-hemolytic, coagulase negative staphylococci in the udder may cause a slight irritation as indicated by a moderate rise in the leucocyte count. In the writers experience and by evidence presented in a later part of this work, even such strains may give rise to an infection exceeding the state of a slight irritation.

Infection of the udder with more virulent strains may lead to a transient or persistant form of mastitis.

In chronically infected cows the leucocyte count exceeds 500,000/cc and small flakes may be present in the milk at infrequent intervals. Usually the chronically

affected animals produce a fair quality of milk. However, enduring infections often lead to induration of the udder and decrease in milk production. Extremely virulent strains of staphylococci may produce acute mastitis that may result in the loss of the animal. There also is the possibility that staphylococci that originally possess little or no pathogenicity may gain entrance to the udder and in time acquire more agressive properties. The writer has examined a large dairy herd in which staphylococci of the same phage type and of the same in vitro reactivity caused mastitis ranging from a slight irritation to the gangrenous form.

(4) Special features of staphylococcal mastitis. According to the foregoing the pathogenic effects of staphylococci do not seem to differ much from those of a number of other agents of bovine mastitis. There are, however, three features which give staphylococci a distinct place among mastitis-causing organisms: their ubiquity, an unusually high degree of variability and the ability to cause a quite severe form of mastitis over a long period of time with little or no visible alterations in the secretion of the invaded gland. A potential pathogen bearing such features is bound to present problems of a very complex nature to those concerned with the control of mastitis. Veterinarian, laboratory worker and dairymen alike have to realize that the danger of an outbreak of epizootic staphylococcal

mastitis is continuously present in every dairy herd. With antibiotic therapy it is possible to eradicate streptococci completely in a herd and with reasonable hygiene streptococci can be kept out of the herd for long periods. However, there is no way to eliminate the staphylococcal population permanently from the animals and their environment. Staphylococcal mastitis may be treated successfully: the clinical symptoms of the disease may disappear, secretion may return to normal after a few days, a microscopic examination will show complete extinction of the udder flora and decrease of the leucocyte count to a normal level, but - contrary to mastitis caused by other organisms - a microscopic examination three weeks after treatment may show that the staphylococcal population has established itself again in the udder. We find here a very interesting parallel to the situation in human communities. Here, also, it seems impossible to protect the individual for any long period of time from infection and reinfection with staphylococci. Strains may be supplanted by others, and degree of virulence may change from time to time, clinical manifestation may be absent for long periods. None of these fluctuations, however, should lead to the disregard of the presence of staphylococci as a potential source of disease. In the management of a dairy herd, therefore, knowledge of the continuous presence of staphylococci, must influence all methods

of sanitation, hygiene and treatment. Disregard will inescapably lead to the vicious circle of an ever recurrent chronic mastitis condition in a considerable part of the herd. "Invisible mastitis" is a term under which this condition is now commonly known to dairymen and it should be realized that its persistance in a herd will do more damage than any acute outbreak.

(5) Sources of Staphylococcal infection on the skin and in the environment of the cow. It has been mentioned before that ubiquity is one of the features that make staphylococci such frequent agents in bovine mastitis. Spencer and Lasmanis (19) present a very complete account on staphylococcal cultures isolated from a herd of sixty-two dairy cows.

(See Table No. II)

TABLE NO. II

Sources of Staphylococcal Infection on the Skin and in the Enviornment of the Cow

Location	No. Examined	Mannite Pos.	Hemolytic	Coagulase Pos.
Skin of teats	248	194	77	12
Hair of flank	62	42	12	0
Vulva	62	12	4	0
Floor beneath Cow	62	47	4	0
Teat cups before milking period	12	0	0	0
Teat cups before disinfection	124	102	47	26
Teat cups after disinfection	124	57	43	15
Hands before milking	2	2	1	0
Hands during milking	4	4	0	0
Disinfectant for teat cups	36	10	6	2
Aseptically collected milk samples	248	52	59	19

Number of samples with microcci positive

The skin of the teats and the teat cups are shown to be the principal extramammary reservoirs for coagulase producing hemolytic staphylococci. Disinfection of teat cups by dipping in two successive pails of sodium hypochlorite solution seemed to be ineffective in many cases for complete elimination of contamination. It was also observed that staphylococci remained alive on dry straw for 49 days. During visits on dairy farms the writer has observed that the evidence given in this table is either unknown or frequently neglected. In a later part of this work, the importance of a proper milking hygiene will be discussed in detail.

Materials and Methods

1. Materials:

(1) Laboratory part: For the laboratory part of this work materials and facilities of the Provincial Dairy Laboratory, Edmonton have been used. The phage typing of 80 strains of staphylococci was performed by the phage typing department of the Provincial Laboratory of Public Health, Edmonton. The Provincial Dairy Laboratory is carrying out routinely a mastitis control program for the Province of Alberta as a free service to the dairy industry. On an average 5,000 quarter samples are sent to this laboratory every year for a bacteriological examination. The samples are taken by the local veterinarians. Sterile vials and shipping containers are provided by the laboratory. The following materials are used in the laboratory diagnosis of staphylococcal mastitis:

a.) Methylene blue stain: used for the staining of milk smears.
0.6 gram certified methylene blue chloride
100 ml. 95 % ethyl alcohol.

b.) Microscope: "Zeiss Standard" binocular
oil immersion objective 100/1.25 0.16 mm.

c.) Oxblood agar medium: 1,000 ml. beef heart infusion
10 grams Bactro tryptose ("Difco")
5 grams Sodium Chloride
15 grams Bacto Agar
50 ml citrated ox blood

Final pH 7.1 - 7.4

d.) Beef heart infusion broth:

500 ml beef heart infusion prepared from fresh beef hearts

5 grams proteose peptone ("Difco")

2.5 grams sodium chloride

Final pH 7.4

e.) Mannitol Broth:

1,000 cc nutrient extract broth

1.0 % mannite

Final pH 7.4

Use bromocresol purple as indicator

f.) Bromocresol purple indicator:

0.5 grams bromocresol purple

100 cc distilled water

g.) Test for coagulase production:

Dehydrated human plasma ("Difco")

h.) Sensitity tests:

Filter paper discs containing various concentrations of the following antibiotics: Erythromycetin, Terramycin, Tetracylcine, Furacin, Chloromycetin, Penicillin, Neomycin and Streptomycin.

(2) Clinical Part. The practical work was carried out either by the writer himself or by persons under his supervision. The materials were provided by the owners of the two dairy farms involved according to the writer's specifications. The examination of samples was performed according to routine standards using

the facilities of the Provincial Dairy Laboratory. For field tests the California Mastitis Test (28) has been employed.

II. Methods:

(1) Collection of Samples: Samples submitted to the laboratory for bacteriological examination should contain only the organisms present in the udder. Therefore every precaution should be taken to prevent contamination from outside sources. The following sampling procedure has been tried successfully by the writer:

a.) Just before milking, the cow's udder is thoroughly washed with a solution of one ounce of 1.6 % Hibitane in 1 quart of warm water.

b.) Thoroughly swab the end of the teats with cotton batting soaked in Hibitane.

c.) Swab the orifice of the teats with cotton batting soaked in 75 % alcohol. Use fresh cotton batting for each teat.

d.) From each teat strip out three to four streams of milk into a strip cup. Report any flakes observed.

e.) From each quarter withdraw a sample of at least 20 ml. into a sterile sample vial. During sampling hold vial in a slanted position to avoid contamination by dirt particles dropping down from the animals body.

f.) Cool the samples immediately and have them delivered to the laboratory as soon as possible.

(2) Milk smears: Smears are made from milk samples which have previously been incubated for 24 hours at 37°C. One wire loop full of the sample is streaked over an area of approximately 1 cm^2 on a glass slide. The smear is then air dried, fixed by flaming, defatted in xylol, and stained in methylene blue chloride.

(3) Cultural methods: In the laboratory portions of 10 ml. of each sample are transferred to sterile culture tubes. To each culture tube 10 drops of bromocresol purple are added. Bromocresol purple is used to indicate alterations in the pH value of the milk. If the pH value is normal "Air Force blue"color is produced. Abnormal acidity in the milk causes yellow and abnormal alkalinity purple color. The tubes are then incubated for 24 hours at 37° C. After incubation color changes, growth, and sediment in the tubes are observed and recorded.

One loop-full of the incubated sample is then streaked onto a glass-slide for microscopical examination.

One loop-full of the fresh milk sample is also inoculated to an oxblood agar plate. The inoculated plates are incubated at 37° C for 48 hours. Any organisms to be identified are then taken from well isolated colonies on the plate and transferred to beef heart infusion broth for further incubation of 24 hours at 37° C.

The purity of the culture is then ensured by microscopical examination.

(4) Methods of Identification of Staphylococci:

a.) Fermentation of mannitol: Two drops of the broth culture are added to 3 ml. of mannitol broth and incubated at 37° C for 24 - 48 hours.

b.) Production of coagulase: One drop of the broth culture is added to 0.3 ml. of rehydrated human plasma and incubated for 1 - 3 hours at 37° C.

c.) Phage typing: the phage types were determined according to the Williams and Rippon system. (25)

d.) Hemolysin production: The production of hemolysins is observed on the oxblood agar plate after incubation times of 24 hours and 48 hours, and again after refrigeration of the culture plates for 24 and 48 hours.

(5) Sensitivity Testing: One loop full of the broth culture is streaked onto a blood agar plate. Blotting paper discs prepared with various antibiotics in low concentration are placed in good contact on the surface of the inoculated blood agar. The plate is then incubated for a maximum of 18 hours at 37° C. The following antibiotic concentrations are used:

Penicillin	2 units
Streptomycin	2 mcg.
Tetracycline	5 mcg.
Furacin (Nitrofurasone)	50 mcg.
Terramycin	5 mcg.
Neomycin	5 mcg.
Chloromycetin (Chloramphenicol)	5 mcg.
Erythromycin	5 mcg.

In reading the results only definite zones of growth inhibition around the individual discs are considered diagnostic for in vitro sensitivity. It is of particular importance in sensitivity testing to keep the incubation time as uniform as possible. The writer has found an incubation time of twelve to fourteen hours at 37° C most reliable. The uniformity of results also seems to depend upon the degree of surface moisture of the medium. Excess moisture seems to dilute the antibiotic concentration below a reliable level and to allow diffusion beyond the desired area of action. Too dry a surface seems to restrict diffusion of the antibiotic to a narrow area around the disc. Therefore, solid media for antibiotic sensitivity testing were not used prior to eight days after preparation and were not stored longer than three weeks in the refrigerator.

RESULTS

I. Laboratory Observations.

(1) Smear Examinations:

(a) Leucocytes and their significance.

On microscopic examination most milk samples will show a dense flora of mixed contaminants. Various bacilli, coliforms, diptheroids, cocci in chain,-cluster-and single pair arrangement are commonly found in large numbers. Many of the organisms are recognized as potential agents of bovine mastitis. It is evident that the presence of these organisms alone does not justify the suggestion of an existing mastitis condition. However, observation of the presence and number of leucocytes in a sample in connection with potential pathogens represents the first step towards the diagnosis of mastitis.

Any leucocyte count over 500,000/ml is considered abnormal (4f)and in connection with a pathogen,proof of an existing mastitis condition.

In cases of severe mastitis, red blood cells, epithelial cells and necrotic material may also be observed.

The number of leucocytes present does not allow any suggestion as to the agent in an individual case. In both staphylococcal

and streptococcal mastitis the writer has made counts of 500,000 to 20 million cells/ml. Of possible value, however, is the observation that in staphylococcal infections, especially in the early stage, large numbers of mononuclear leucocytes are found, while in streptococcal infections, right from the onset, the polymorph type prevails.

(b) Size of bacterial cells: It has been frequently observed by the writer that a connection between the size of the bacterial cell and the degree of pathogenicity exists. In most smears showing large numbers of leucocytes the staphylococcal cells were smaller than 1 micron in diameter. Cells of medium or large size were either found in connection with mild infections or as common contaminants. It also has been observed that with decrease in cell size the typical grape-like arrangement becomes more pronounced. Larger cells seem to have a tendency to lump together in irregular bulky clusters.

(c) Number of bacterial cells: The number of bacterial cells in smears from staphylococcal mastitis seems to be considerably lower in severe infections than in milder cases. Numerous smears have been examined showing leucocyte counts of 5×10^6 and more in

which the staphylococcal agent was found only sporadically in form of small patches of 3 - 6 cell - pairs. In mild infections usually large numbers of single cells and cell clusters could be observed.

No satisfactory explanation for this observation could be found. It may be that the foci of more virulent strains are situated deeper in the host tissue and the organisms are, therefore, shed in smaller numbers. Also the rapid and violent defense reaction of the host may succeed in keeping the number of bacterial cells down.

(d) Discussion of smear examination and diagnostic value: It is possible for an experienced observer to establish a definite diagnosis of mastitis by microscopic examination alone. Clinical, cultural and biochemical evidence is of a more or less confirmatory character. Of great importance for the microscopic diagnosis and the identification of the agent is, in the writers experience, the phenomenon of phagocytosis.

As shown below, there may be various reasons of a non-infectious nature for the presence of leucocytes in milk samples. In these cases the abnormal number is due to a mechanical or traumatic damage to the tissue barrier between the inside of the gland and

the

circulatory system, and has nothing to do with the release of leucocytes as a host defense against parasites. In infectious diseases the release of leucocytes is directed specifically against the parasite and aimed at its destruction. This specificity is probably brought about by concurrent antigen-antibody interaction; the parasite acting antigenically on the host system is counteracted by the release of specific antibodies by the host. Phagocytes and antibodies function in close association. In the presence of antibodies, the bacterial cells are ingested by phagocytes at a much faster rate than when antibodies are absent (29a) The specifity of the antigen-antibody reaction accompanying phagocytosis can be observed in smears from mastitis and this observation may be suggestive of the identity of the agent. As mentioned before, in many milk smears a variety of organisms is found and a number of them may be potential pathogens. The microscopic observation that one particular organisms is phagocytized while others are omitted makes it suggestive that this organism is the agent of the infection.

Particularly in connection with the microscopical diagnosis of staphylococcal mastitis it seems important to mention the possible sources of error: As stated before, staphylococci are found commonly in milk samples; thus fulfilling the postulate of the presence of a potential pathogen in many instances. The emphasis in the diagnosis had further been placed on the presence and number of leucocytes. It is of outmost importance to eliminate all other causes for abnormal numbers of leucocytes before suggesting an infectious process:

i.) In the early lactation state leucocytes frequently are found in larger numbers. It is therefore important that the lactation history of the animal is known to the laboratory worker.

ii.) Mechanical defects of the milking machine or overmilking (careless continuation of milking after milk flow has ceased) may be the cause of an increased leucocyte count.

iii.) External or internal injuries of the udder may be the reason for the presence of leucocytes. Blows to the udder or injuries of the teats are frequent incidents in dairy herds.

iv.) During the examination of almost every dairy herd several smears are found which show an abnormally high leucocyte count and in which the bacterial flora is completely absent. Such smears are highly suggestive of recent treatment of the particular quarter, and do not allow any conclusions as to the success of this treatment. Therefore, no samples should be submitted for examination for a period of at least three weeks after treatment.

(2) Observations on 80 strains of staphylococci isolated as agents from cases of bovine mastitis:

For the purpose of this work eighty strains of staphylococci have been isolated and tested. All strains were found, beyond doubt, to be the agents in particular cases of mastitis. The samples from which the strains were isolated showed leucocyte counts of 1,000,000 or more per ml.

TABLE No. III

Morph. on Ox-BAP	Hemolysin	Mannitol	Coagulase	Phage Type	Sensitivity
1. Grey, large, flat, dry, regular	alpha beta	+	+	7/42e/73/77/81/42d	E, C, N, Tt, Te, St, F.
2. Grey, large, flat, dry, regular	alpha beta	+	+	81	E, C, N, Tt, Te, St, F
3. Cream, small, convex, smooth, regular	alpha beta	+	+	42d	E, C, N, Tt, Te, St, F, P
4. Grey, medium, convex, rough, regular	beta	+	+	6/7/47/42e/70/73/75/77/81/42d	E, C, N, Tt, Te, St, F, P
5. White, medium, flat, smooth, regular	none	+	-	42d	E, C, N, Tt, Te, St, F
6. Tan, small, convex, smooth, regular	alpha	+	+	42d	E, C, N, Tt, Te, St, F
7. White, medium, flat, smooth, regular	alpha	+	+	42d	E, C, N, Tt, Te, St, F, P
8. Cream, large, flat, dull, regular	beta	+	-	42d	E, C, N, Tt, Te, St, F, P
9. Cream, large, flat, dull, regular	beta	+	-	42d	E, C, Tt, Te, St, F

Morph. on Ox-BAP	Hemolysin	Mannitol	Coagulase	Phage Type	Sensitivity
10. White, medium, flat, smooth, regular	alpha delta	+	+	42d	E, C, Tt, Te, St, F, P
11. White, medium, flat, smooth, regular	alpha beta	+	+	81	E, C, Tt, Te, St, F, P
12. White, medium, flat, smooth, regular	alpha beta	-	+	79/36/6/7/47/53/54/42e/73/81	E, C, N, Tt, Te, St, F, P
13. White, medium, flat, smooth, regular	alpha beta	+	+	79/6/7/47/54/42e/73/81	E, C, N, Tt, Te, St, F
14. Tan, large, flat, smooth, regular	beta	+	-	81	E, C, N, Tt, Te, St, F, P
15. White, medium, flat, smooth, regular	none	+	-	81	E, C, N, Tt, Te, St, F, P
16. White, medium, flat, smooth, regular	alpha	+	+	42d	E, C, N, Tt, Te, St, F, P
17. Yellow, small, convex, smooth, regular	beta	+	+	N. T.	E, C, N, Tt, Te, St, F, P
18. White, medium, flat, smooth, regular	alpha	+	+	42d	E, C, N, Tt, Te, St, F

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]		[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]		[illegible]	[illegible]
[illegible]	[illegible]			[illegible]	[illegible]

Morph. on Ox-BAP	Hemolysin	Mannitol	Coagulase	Phage Type	Sensitivity
19. Golden, medium, convex, smooth, regular	beta	+	+	42d	E, C, N, Tt, Te, St, F
20. White, medium, flat, smooth, regular	alpha	+	+	42d	E, C, N, Tt, Te, St, F
21. White, medium, flat, smooth, regular	alpha	+	+	42d	E, C, N, Tt, Te, St, F, P
22. White, medium, flat, smooth, regular	alpha	+	+	42d	E, C, N, Tt, Te, St, F, P
23. Cream, medium, flat, smooth, regular	alpha	+	+	42d	E, C, N, Tt, Te, St, F, P
24. Yellow, small, convex, smooth, regular	beta	+	+	3A	E, C, N, Tt, Te, St, F
25. White, medium, flat, smooth, regular	none	+	+	N. T.	E, C, N, Tt, Te, St, F, P
26. White, medium, flat, smooth, regular	alpha	+	+	42d	E, C, N, Tt, Te, St, F, P
27. Yellow, small, convex, smooth, regular	beta	+	+	81	E, C, N, Tt, Te, St, F, P

Morph. on Ox-BAP	Hemolysin	Mannitol	Coagulase	Phage Type	Sensitivity
28. Cream, small, convex, rough, irregular	alpha delta	+	+	73	E, C, N, Tt, Te, St, F.
29. Golden, medium, flat, smooth, regular	beta	+	+	42d	E, C, N, Tt, Te, St, F
30. Tan, small, convex, smooth, regular	delta	+	-	N. T.	E, C, N, Tt, Te, St, F
31. Cream, small, flat, dull, regular	beta	+	-	42d	E, C, N, Tt, Te, St, F
32. White, small, convex, smooth, regular	alpha beta	+	-	42d	E, C, N, Tt, Te, St, F, P
33. Golden, medium, convex, irregular	none	+	-	42d	E, C, N, Tt, Te, St, F
34. Grey, large, flat, dull, irregular	alpha beta	+	+	42d	E, C, Tt, Te, St, F
35. Grey, large, flat, smooth, regular	beta	+	+	47/53/54/75	E, C, Tt, Te, St, F
36. Grey, very large, convex, wet, regular	alpha beta	+	+	42d	E, C, Tt, Te, St, F

Morph. on Ox-BAP	Hemolysin	Mannitol	Coagulase	Phage Type	Sensitivity
37. Grey, small, convex, smooth, irregular	delta	+	+	42d	E, C, Tt, Te, St, F
38. Grey, small, flat, smooth, regular	beta	+	+	42d	E, C, Tt, Te, St, F, P
39. Golden, small, convex, smooth, regular	alpha beta	+	+	42d	E, C, Tt, Te, St, F, P
40. Cream, small, convex, smooth, regular	alpha beta	+	+	6/7/47/54/42e/73/77/81/42d	E, C, Tt, Te, St, F, P
41. Grey, small, flat, smooth, regular	Beta	+	+	42d	E, C, N, Tt, Te, St, F, P
42. Cream, small, convex, smooth, regular	alpha beta	+	+	42d	E, C, N, Tt, Te, St, F, P
43. Grey, large, flat, smooth, irregular	beta	+	+	42d	E, C, N, Tt, Te, St, F, P
44. Golden, medium, convex, smooth, regular	beta	+	+	52/52A/81	E, C, N, F
45. Cream, small, convex, wet, regular	delta	+	+	N. T.	E, Tt, Te, P

Morph. on Ox-BAP	Hemolysin	Mannitol	Coagulase	Phage Type	Sensitivity
46. Golden, small, convex, smooth, regular	beta	+	+	N. T.	E, C, N, Tt, Te, F
47. Tan, small, convex, smooth, regular	alpha	+	+	N. T.	E, C, Tt, Te, F, P
48. Yellow, small, convex, rough, regular	beta	+	-	42d	E, C, Tt, Te, St, F, P
49. Golden, small, convex, smooth, regular	beta	+	+	81	E, C. Tt, Te, St, F, P
50. Grey, large, flat, smooth, regular	alpha	+	+	29/79/3A/3B/55/6/7/47/53/54/42e/73/77/81	E, C, N, Tt, Te, F, P
51. Cream, small, convex, smooth, regular	alpha delta	+	+	29/6/7/47/54/42e/70/73/77/81/42d	E, C, Tt, Te, F, P, St
52. Grey, small, convex, smooth, irregular	beta	+	+	52/3b/3c/6/47/54/42e/75/81	E, C, N, Tt, Te, F, P
53. Cream, flat, small, smooth, regular	beta	+	+	29/79/3a/3b/6/7/47/54/42e/70/73/75/77/81/42d	E, C, N, Tt, Te, F
54. Cream, large, flat, smooth, regular	beta	+	+	53/81/42d	E, C, N, Tt, Te, F, P, St

Morph. on Ox-BAP	Hemolysin	Mannitol	Coagulase	Phage Type	Sensitivity
55. Cream, large, flat, smooth, regular	beta	+	+	53/81/42d	E, C, N, Tt, Te, F, P, St
56. Yellow, large, flat, smooth, regular	alpha beta	+	+	N. T.	E, C, N, Tt, Te, St, F, P
57. Cream, large, flat, smooth, regular	beta	+	+	3a/3b/55/6/7/47/53/54/42e/70/73/81	E, C, Tt, Te, St, N, F
58. Grey, large, convex, smooth, irregular	alpha beta	+	+	81	E, C, Tt, Te, N, F
59. Grey, small, convex, smooth, regular	beta	+	+	N. T.	E, C, Tt, Te, N, St, P, F
60. Grey, medium, flat, smooth, regular	none	-	-	N. T.	E, C, Tt, Te, N, St, P, F
61. Grey, medium, flat, smooth, regular	none	+	+	N. T.	E, C, Tt, Te, N, St, F
62. Grey, large, flat, smooth, regular	alpha beta	+	+	N. T.	E, C, Tt, Te, N, St, F
63. Cream, large, flat, smooth, regular	beta	+	+	42d	E, C, Tt, Te, N, St, F, P

Morph. on Ox-BAP	Hemolysin	Mannitol	Coagulase	Phage Type	Sensitivity
64. Grey, medium, flat, smooth, irregular	beta	+	+	81	E, C, Tt, Te, N, St, F, P
65. Cream, medium, convex, smooth, regular	beta	+	+	42d	E, C, Tt, Te, N, St, F, P
66. Grey, medium, flat, smooth, regular	beta	+	+	54/81	E, C, Tt, Te, N, P.
67. Grey, large, flat, smooth, regular	beta	+	+	N. T.	E, C, Tt, Te, F, P
68. Grey, medium, convex, smooth, regular	beta	+	+	N.T.	E, C, N, Tt, Te, F, P
69. Cream, small, flat, smooth, regular	alpha beta	+	+	42d	E, C, N, Tt, Te, St, F, P
70. Cream, large, flat, smooth, regular	alpha beta	+	+	N. T.	E, C, N, Tt, Te, St, F, P
71. Orange, small, convex, smooth, regular	alpha beta	+	+	42d	E, C, N, Tt, Te, F, P
72. Golden, medium, convex, smooth, irregular	beta	+	+	42d	E, C, N, Tt, Te, F

Morph. On Ox-BAP	Hemolysin	Mannitol	Coagulase	Phage Type	Sensitivity
73. Grey, medium, convex, dull, regular	alpha beta	+	+	29/52A/79/3A/3B/55/6/7/47/53/54/42e/70/73/81	E, C, N, Tt, Te, F, P
74. Grey, medium, flat, smooth, irregular	alpha beta	+	+	29/52A/79/3A/3B/55/6/7/47/53/54/42e/70/73/81	E, C, N, Tt, Te, F, P
75. Cream, large, flat, smooth, regular	alpha beta	-	-	81/42d	E, C, Tt, Te, N, St, F, P
76. Yellow, small, convex, smooth, regular	beta	+	+	29/52/52A/6/7/47/54/42e/73/77/81/42d	E, C, Tt, Te, N, St, F
77. White, large, convex, smooth, regular	alpha	+	+	N. T.	E, C, Tt, Te, N, St, F, P
78. White, large, convex, smooth, regular	alpha	+	+	N. T.	E, C, Tt, Te, N, St, F, P
79. Grey, large, convex, smooth, regular	alpha	+	+	29/52A/6/7/47/54/42e/73/77/81	E, C, Tt, Te, N, F, P
80. Cream, small, flat, smooth, regular	alpha beta	+	+	29/52A/6/7/47/54/42e/73/77/81	E, C, Tt, Te, N, F, P

(3) Discussion of cultural observations:

a.) Colonial morphology: The pigment produced by various species of staphylococci has played a certain role in the nomenclature. *Staph. aureus*, *Staph citreus*, *Staph. albus* are terms still quite commonly used to designate certain species(32)In the case of *Staph. aureus* golden pigmentation has long been regarded as the main differential character of the species. Within the scope of this work the color-criterion has been found of very little significance in the identification of staphylococci. As table No. III shows, a large variety of pigmentation has been observed including all shades of yellow from deep orange to light cream and from grey to pure white. Some strains did not produce any pigment and appeared as greyish-opaque colonies. It was not possible to establish any relationship between color production and the other properties of individual strains nor was there any evidence for the common identity of strains with similar pigmentation. The same holds true for the other cultural characteristics. They too were subject to such a wide scale of variations that it seems impossible to select any of them as typical. Colonies of the same phage type and age showed such contrasting features as: flat and raised, circular ridge and no ridge, regular and irregular margins, smooth and rough surface texture shiny and dull, viscid and dry. One of the most striking observations was the variation in colony size. In some cases strains of the same phage type, grown under the same conditions produced colonies ranging in diameter from 2 mm. to more than one-quarter of an inch. Thus the colonial morphology of

Staphylococcus aureus, although regularly helpful in preliminary study, is so frequently subject to gross variation that it can never be considered entirely dependable in species identification.

b.) Production of hemolysins: The productionof one or more hemolysins by staphylococci has long been considered a decisive factor in the distinction between so-called "mastitis staphylococci" and those belonging to a hamless, normal udder flora. Table No. IV shows the incidence of hemolysins in the eighty strains subject to these observations.

TABLE NO. 1V

Hemolysins Produced by Mastitis Staphylococci

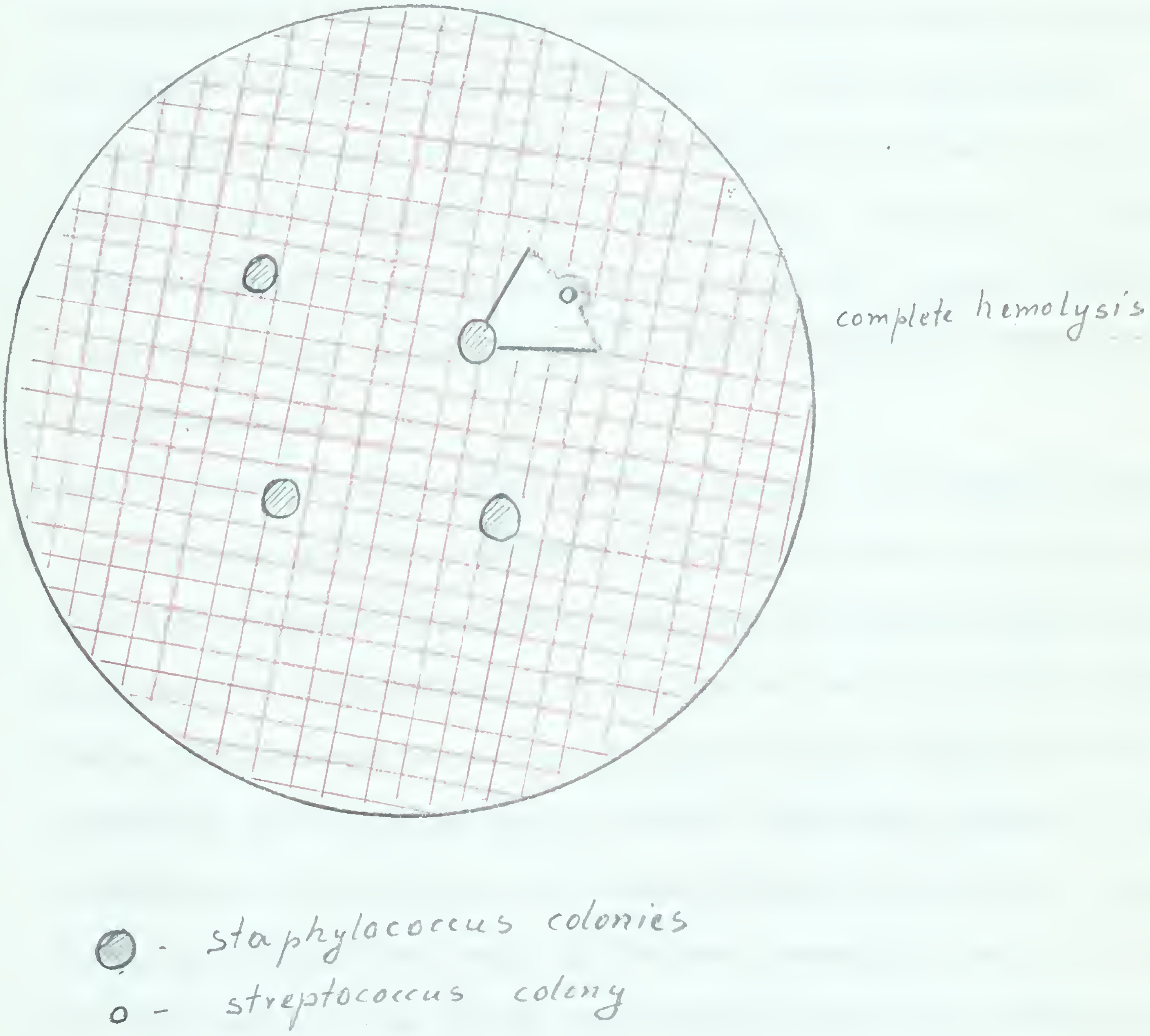
Strains	Alpha Only	Beta Only	Delta Only	Alpha Beta	Alpha Delta	Beta Delta	Alpha Beta Delta	No Hemolysins
80	13	32	3	22	3	0	0	7

The various hemolysins were determined according to their action on the red blood cells of the ox. Ox red blood cells are lysed by alpha, beta, and delta hemolysins (25b) A specific hemolytic pattern is produced on ox blood agar plates by the individual hemolysins. Although sheep and rabbit red blood cells seem preferable in the identification of hemolysins, ox blood was used in this specific case as recommended by Minnett (12) and Plastridge, et al. (4g)

It must be remembered in this connection that these findings were obtained by the plate technique using bovine red blood cells. This technique offers reliable results only if the plates are examined verycarefully. There is always the danger that the presence and action of one hemolysin is masked by that of others. This holds true especially with regard to the detection of delta hemolysin. Its very restricted zone of action may be masked by a wide zone alpha-effect. Also the alpha-effect may be suppressed by beta action. It is therefore necessary to read the plates as soon as possible after the first signs of hemolysin production have appeared. The writer recommends a first examination after twelve hours of incubation and from then on readings at intervals of three to four hours.

The results confirm the findings of Elek and Levy (20) to the extent that the frequency of beta hemolysin production is generally great in animal strains. Bryce and Rountree (21) report that bovine staphylococci in particular were found to be frequent producers of beta hemolysin. Surprisingly low in comparison to the findings of Elek and Levy (20) seems the percentage of delta hemolysin producing strains. William and Harper (22) found that this particular lysin acts on human, monkey, horse, rat, mouse, sheep, rabbit and guinea pig red blood cells. Marks and Vaughan (23) have found that bovine albumin and normal horse serum diminish the action of delta lysin. These findings may offer an explanation for the fact that delta producing staphylococci are not represented

more frequently in the table of 80 known mastitis agents. Although evidence suggests that most of the staphylococcal agents of bovine mastitis produce hemolysins, there still remains a number of strains, apparently non-hemolytic, which are capable of the same pathogenic affects. This fact depreciates hemolysis as a criterion of pathogenicity in strains recovered from bovine mastitis. Observations suggest that the ability to produce hemolysins - like colony morphology - is subject to variation. On numerous occasions the writer has found evidence that the production of hemolysin is delayed or the inherent ability to produce lysins may not become obvious at all unless an interaction of diffusible substances from other organisms takes place. One of these interacting substances seems to be produced by streptococci: Staphylococci and streptococci are frequently found as combined agents of bovine mastitis. In typical cases a dense flora of staphylococci and streptococci will be observed on direct culturing of the milk samples on ox-blood agar plates. In such instances it has quite frequently been observed that hemolysis was produced only by those staphylococcal colonies which grew in the close neighbourhood of alpha-hemolytic streptococci. The shape of the zone of clearing differed markedly from the usual concentric pattern around the colony. It appeared as a sharply margined segment of 45 - 90 degrees, the open side turned towards the streptococcus colony, with the staphylococcus colony being the converging-point, (See illustration) The mathematical accuracy guiding this effect is illustrated by the observation that the streptococcus colony had its geometric position exactly on the line dividing the


complete hemolysis
▨ - staphylococcus colonies
o - streptococcus colony

segment in half. The rest of the circle, outside the segment, around the staphylococcus colony, did not show any trace of hemolysis. This effect of interaction can similarly be observed in direct cultures of samples from animals suffering from a combined staphylococcus-alpha-streptococcus infection in one quarter and from a pure staphylococcus infection caused by the same strain in another. In numerous instances the culture from the pure staphylococcus infection showed no trace of hemolysin production. On the other hand, especially if the streptococcus population greatly outnumbered the staphylococcus population, the area of inoculation with material from the combined infection showed complete clearing, thus entirely masking the individual effects of streptococci.

The observations on this masked ability to produce hemolysin have led to this experiment: Staphylococcus colonies which had not produced hemolysins on direct culturing and which had not been exposed to the action of streptococcal lysins were subcultured to ox-blood agar plates, together with colonies of the same strain, which had been exposed to the effects of streptolysin and had produced hemolysin. As a control an ox-blood agar plate was inoculated with the milk of the sample from which the staphylococci and streptococci had previously been isolated. After incubation for fourteen hours at 37° C all colonies had produced hemolysis of the same degree and kind. The control showed that only staphylococcal colonies growing in close neighbourhood to streptococcal colonies produced a segment shaped hemolysis while isolated colonies did not produce any hemolysis.

This experiment led to the suggestion that the factor suppressing the development of hemolysin is of an external nature and may be carried by the milk of the infected quarters. A loop full of this milk is used in direct cultuning. The evidence also suggests that some diffusible product originating from alpha streptococci is capable of neutralizing this suppressing factor in a limited area, thus potentiating the action of staphyloccus hemolysins. If this hypothesis holds true then it might have some bearing on the *in vivo* situation during the course of an infection. It is known that staphylococcal mastitis often follows a primary streptococcal infection. Perhaps streptococci may potentiate the full pathogenic effect of staphylococci *in vivo* just as they potentiate their hemolytic effect *in vitro*.

Another hemolytic phemomenon frequently observed by the writer is the potentiation of beta hemolysin by refrigeration. The knowledge of this phenomenon can be put to practical use in the cultural routine in instances where only a slight discoloration of the medium can be observed. This discoloration may be mistaken for the clearing effect which milk sometimes exerts on blood agar. If the doubtful plates are placed into the refrigerator over night the presence of beta hemolysin will result in a complete clearing of zones around the colonies and sometimes in a pronounced ring effect.

The capacity, however, of a strain of staphylococcus to produce hemolysis can not be regarded as an index of its actual or potential pathogenicity. Hemolytic staphylococci are quite frequently isolated from normal udders and some non-hemolytic strains are also able to cause acute mastitis.

c.) Fermentation of Mannitol: This biochemical test is important because of theexperience that mannitol belongs to the carbohydrates most consistently fermented by staphylococci. Further evidence (cited by Elek) shows that virtually all toxigenic strains give positive mannitol fermentation reactions while the great majority of non-toxigenic strains is negative. The results of the test on the 80 pathogenic strains confirm this conclusion. Only three of the strains were mannitol negative. However, the writer has found that at least ten percent of non-hemolytic, coagulase negative staphylococcus strains isolated from normal udders are capable of fermenting mannitol. This observation suggests that the fermentation test is of subordinate significance and only useful in connection with other diagnostic evidence.

d.) Production of coagulase: Of the 80 strains tested, 69 produced coagulase. The 11 remaining strains were considered coagulase negative only after each had been tested with a different lot of plasma, and the initial finding had been confirmed. The ability to produce coagulase is widely accepted as a definite proof of virulence and often the test on the presence of coagulase is considered conclusive in establishing pathogenicity. It is believed that staphylocoagulase exerts its effect in two ways:

i.) Hale and Smith (24) were able to demonstrate that coagulase delayed phagocytosis. Thus the organisms eluded the first line ofdefence of the host.

ii.) After the lesions are formed by the action of other factors coagulase plays a protective role by initiating fibrin deposits around the site of the lesion

In the light of these theories the eleven coagulase negative mastitis strains become of particular interest: If coagulase is recognized as a factor favouring initial invasion then the coagulase negative pathogenic strains must possess other means of overcoming the first defense reactions of the host. Coagulase may add to the virulent character of a given strain but evidence suggests that it is not the all important factor and that the pathogenicity of the strain is not dependant upon the production of coagulase.

For the purpose of classification of staphylococci the character of coagulase production is of greater reliability than e.g. the production of hemolysins. The following personal observation suggests that the ability to produce coagulase or the failure to do so is a characteristic with little tendency to vary: Three coagulase positive and three coagulase negative strains were isolated, from the normal quarters of a dairy herd. The same strains were isolated six months later from the same herd as agents of bovine mastitis. The ability or failure to produce coagulase had been retained unchanged.

Prolonged culturing on artificial media did not seem to influence the characteristics of coagulase production: Ten coagulase positive and ten coagulase negative strains of staphylococci were subcultured four times. In the first two transfers beef heart infusion broth was used as a medium. For the two other transfers ox-blood agar and nutrient agar were chosen. Coagulase tests were carried out after each subculturing. The last cultures on nutrient agar were then kept for twenty days in the refrigerator and a re-test was

done after this period. All ten initially negative strains remained negative. All ten initially positive strains retained this characteristic.

From the evidence presented in this paragraph it may be concluded that it is fairly safe to classify staphylococci into a coagulase positive and a coagulase negative group. However, at least as far as bovine mastitis is concerned, it would be misleading to attribute potential pathogenicity only to the coagulase producing strains.

e.) Phage typing: In human bacteriology phage typing has become an important method of fine classification of staphylococci. It emerged from the search for an identity pattern independent of factors subject to frequent variation or of properties allowing only a division into large groups. Therefore, the strain-specifity of staphylococcal bacteriophage was used to identify and recognize individual strains of staphylococci. It must be visualized that phage typing is strictly a labelling procedure and has nothing to do with the metabolic, biochemical or pathogenic properties of a given strain. The full importance of a reliable typing system becomes obvious in staphylococcal infections assuming epidemic or epizootic characteristics. There is strong evidence that staphylococcal mastitis, by the agency of highly virulent strains, spreads not only among animals within individual farm limits but is also carried over to other herds. In such instances it is of great advantage to recogniz and follow up a particular strain. The value of the phage typing system for the control of staphylococcal mastitis is illustrated by the following findings: For three Alberta

dairy herds a phage type pattern of the staphylococcal population was established by one investigation. Within a period of nine months two further investigations were carried out on each of the herds. The pattern of two of the herds remained unchanged. In the third herd one new staphylococcal strain was discovered during the second investigation. Within four weeks this new strain became the agent of a minor flare-up of mastitis in the herd. This suggested either the introduction of a more virulent strain or a strain to which the animals possessed no herd immunity, but in either case confirmed the value of phage typing as an epidemiological tool. The above series of examinations also brought to light interesting evidence on the stability and persistance of individual phage types.

An observation of a more incidental character also confirmed the persistence of the phage type: One herd had been under continuous observation for the past eighteen months. During a mastitis flare-up, in the first two months of this period a staphylococcus strain has been isolated which was found to be of Type 42 D. The colonies were large, flat, circular, golden and produced beta hemolysin. Of special interest was that this particular strain showed a high degree of resistance to the action of tri-cresol used as a bactericide in the preparation of an autogenous vaccine. Twelve months later another mastitis out-break occurred and as one of the agents a staphylococcal strain was isolated, again of the 42D type. The colonies were small, raised, cream, colored, moist and exhibited only weak hemolysin production. But this strain was

again the only staphylococcus strain in the herd exhibiting a high resistance to tri-cresol. In several hundreds of strains employed in the preparation of bacterin the writer has found only two strains of different origin showing such a strong resistance to bactericides. Thus it may be concluded that this property is fairly rare amongst animal strains.
It seems, therefore, highly probable that on the two occassion mentioned the same strain had been isolated. Colony morphology and hemolysin production had under-gone variation during the period of twelve months but one outstanding characteristic had remained unchanged to confirm the persistance of the original strain and the permanance of its phage type.
It has been said that phage typing is strictly a labelling procedure and that the phage number assigned to a particular strain has no constant significance with regard to pathogenicity or other properties. Yet it may be of interest to know the frequency of certain phage types as agents in bovine mastitis.
Such purely empirical evidence - obtained over a long period of time - may contribute to a better knowledge and recognition of micrococci possessing a definite pathogenic potential with regard to mastitis. Table No. V shows the incidence of different phage types in the eighty mastitis strains. In so far as these eighty strains can be considered representative, the types 42D and 81 seem to be the most common as agents of staphylococcal mastitis in this area. The majority of the polyvalent strains, that is strains lysed by varied groups of phages, reacted in addition to phage 81 or to both 81 and 42D. (Compare to Ref.No.33)

TABLE NO. V

Incidence of Phage Types

No. of Strains	42 d	81	3 A	73	54/81	81/42 d	Polyvalent	Not Typable
80	33	8	1	1	1	1	19	16

One observation is of special interest: both coagulase-positive and coagulase-negative strains were found among the staphylococci identified above as Types 42 d and 81. These strains had been accepted as virulent from the knowledge of the circumstances of their isolation; their phage types were found to be those most frequent in mastitis. (So here at least coagulase production was perhaps less reliable than phage typing as a guide to pathogenicity.)

f.) <u>Sensitivity to various antibiotics.</u> The introduction of antibiotics into the therapy of bacterial diseases has opened a practical field of a very complex nature. Miraculous success and unexpected failure seem to accompany every step in this field. This especially holds true with regard to staphylococcal infection. The organism has an inherent ability and tendency to vary and mutate and exhibits an unequalled skill in eluding the action of antibiotics. An excellent demonstration of these characteristics is given in bovine mastitis. In the therapy of this disease the extensive and very often unreasonable use of antibiotics may have succeeded in down-grading streptococci as principal agents but it also has contributed very much to the creation of the so-called "staphylococcus problem" in the etiology of bovine mastitis. Elek (25) defines the action of antibiotics as follows: "The action of an antibiotic is selective, some organisms being affected and others not at all or only to a limited degree; each antibiotic is thus characterized by a specific antimicrobial spectrum." Failure to appreciate this may well have led to the current difficulties with anti-

biotic resistance.

The phenomenon of resistance to the actionof antibiotics is explained by several theories. One of them suggests that exposure to subinhibitory concentrations of a drug builds up a gradual increase in tolerance resulting in complete adaptation to the drug. Demerec (26) gives experimental evidence that resistance to penicillin is not introduced by the drug but originates spontaneously through genetic changes. He demonstrated that a sensitive population of staphylococci is in fact composed of individuals with varying sensitivity, such individuals with a low degree of sensitivity are able to survive certain antibiotic concentrations and give rise to a new population. If this new population is exposed to a higher concentration of the drug, again some individuals survive. In this way, a gradual selection takes place and may result in extremely resistant strains. Barber (27) even has described a penicillin-dependent variant, capable of growing only in the presence of penicillin. The alarming increase of resistant staphylococci in human medicine has led to the search of possible reasons. During these investigations it has been found that endemic foci have developed in hospitals. From hospitals resistant organisms have spread into the population in the nasal flora of dischargedpatients. Personal observations suggest that there may be a parallel between these findings and the state of affairs in dairy herds. Every dairy herd represents in a certain sense a closed community. This community harbours in the udder flora a steady pool of staphylococci. These organisms will be exposed to the action of antibiotics quite frequently because the preventive and therapeutic use of these drugs has become

common practice in maintaining animal health. If it is true that the emergence of resistant strains is mainly due to therapeutic failure or to exposure of the organisms to sub-inhibitory concentrations of the drug then many dairy herds must represent ideal breeding places for such strains. There is evidence that in fact the number of resistant strains of staphylococci has increased rapidly in Alberta dairy herds. A wide spread increase of penicillin, streptomycin and sulphonamide resistant strains has already led to a restricted use of these drugs in the therapy of staphylococcal mastitis. Even terramycin, advertised as a "wonder-drug" in mastitis therapy is rapidly loosing its value. Only recently the writer has isolated from an acute outbreak of mastitis a strain of staphylococcus which showed in vitro resistance to chloromycetin, neomycin, streptomycin, penicillin, terramycin, tetracycline and which was sensitive only to erythromycin. If this trend continues than many dairy herds may in fact become endemic foci of resistant strains of staphylococci thus representing a serious public health problem.

In this connection it may be mentioned that in the Province of Alberta, a number of municipalities have no by-law yet enforcing pasteurization of milk sold to the consumer, and also that some strains of staphylococci seem to be able to survive pasteurization and other commercial sterilization processes (4h,7, 30, 31)

In the assay of sensitivity to various antibiotics on the eighty strains under consideration only the results of the tests with low concentrations were considered of therapeutic significance. In the writer's opinion the result of the low concentration in vitro test approaches closest the situation in vivo. In most cases the treatment of mastitis is performed by udder infusion of antibiotics in an oily base. The anatomy of the udder is such that an even distribution of the drug

in all parts of the gland cannot be expected. Thus organisms with even a moderate degree of resistance may survive treatment if they are located in a part of the gland which is not reached by the full concentration of the drug. Table No. VI summarizes the results of the sensitivity tests using various antibiotics:

(4) Further Laboratory Observations:

a.) The coexistence of different pathogenic strains of staphylococci within the same herd and even within the different quarters of the same udder has been observed on several occasions. From one herd five different phage types were isolated as agents of mastitis. Two of the types showed resistance to penicillin, two to penicillin and neomycin, and one was sensitive to all test antibiotics. On another occasion three more morphologically different strains were isolated from three mastitic quarters of the same animal. Two of the strains were of the same phage type, 42 D, while the third strain was of Type 81. The two 42 D strains exhibited resistance to penicillin while the 81 strain was sensitive to all test antibiotics. The coexistence of various strains in a herd increases the difficulties with respect to in vitro sensitivity testing and treatment. Ideally every strain isolated from a mastitic quarter should be individually tested and therapy accordingly adjusted. Certainly any generalization of principles based on insufficient evidence in dealing with staphylococci could lead to error and failure.

b.) The sensitivity of staphylococci to antibiotics compared to that of streptococci: Although many species of streptococci

TABLE No. VI

In vitro Sensitivity to Various Antibiotics (Low Concentrations)

Strains	Sensitive to all Antibiotics Tested	Sensitive to All Test Antibiotics Except :							
		P	N	St	P, N	P, St	St, N	Te,Tt,St,P	N,St,F,C
80	32	18	8	8	5	4	3	1	1

Test Antibiotics

E - Erythromycin
C - Chloromycetin
N - Neomycin
Tt - Tetracycline

Te - Terramycin
St - Streptomycin
F - Furacin
P - Penicillin

have been isolated by the writer and identified as agents in bovine mastitis, none of them has exhibited in vitro a resistance to antibiotics comparable to that of numerous strains of staphylococci. Some streptococcus strains, particularly of the fecalis type, have been found to be resistant to streptomycin in low in vitro concentrations. This was the only antibiotic to which streptococcal resistance was observed. Even more striking was the difference in in vivo sensitivity as expressed by therapeutic results. Most cases of streptococcal mastitis showed a dramatic response to treatment with any drug to which in vitro sensitivity had been established. Microscopic examinations on samples from quarters under treament gave evidence of the lethal effects of the drug. The chains of bacterial cells were found to break up into short pieces or disorganized clumps. Distorted and ruptured cells were numerous. The experience in many cases of staphylococcal infection was quite different. Frequently the drug selected according to in vitro results did not show the expected effect in vivo. After a short response of two or three weeks a relapse occurred and continuation of treatment with the same drug proved completely ineffective. In some cases treatment was continued with a different drug and still the infection persisted stubbornly. On other occasions, although a lasting clinical improvement was achieved, the organisms still could be found in smears associated with an above-normal leucocyte count, five or six weeks after treatment. Microscopic examination, even during treatment, showed that the organisms, although decreased in number, showed no physical damage or abnormality.

This evidence suggests that the ability to produce drug resistant mutants is greater in staphylococci than in streptococci. The microscopical examination during treatment also suggests that the individual staphylococcus cell seems to be able to protect itself better against the damaging effects of drugs than the streptococcal cell. Elek (25) mentions that fibrin deposits on the surface of the cell derived from host sources, may offer this protection.

c.) Resistance of two strains to phenols. In the preparation of autogenous staphylococcal vaccines the writer uses tricresol in a dilution of 0.5 % routinely for sterilization. Two strains of staphylococci, isolated from different cases of mastitis, showed a remarkable resistance against the bactercidal action of the disinfectant. The procedure followed was this: enough of emulsified tricresol is added to a liquid culture to give a final tricresol concentration of 0.5 %. The mixture is shaken thoroughly and then incubated for forty-eight hours at 37° C. During incubation the mixture is shaken frequently. After incubation portions of the mixture are transferred to ox-blood agar plates and to heart infusion broth and reincubated for at least twenty-four hours at 37° C. On the two occasions mentioned subculturing showed that large numbers of viable organisms were present in the mixture. The concentration of tricresol was now doubled and the mixture reincubated for forty-eight hours. On reculturing again large numbers of organisms proved to be alive. The procedure was carried on up to the limit of a two and a half percent cresol concentration in one case and a two percent concentration in the other before complete sterilization could

be demonstrated.

During the whole procedure the bacterial population kept multiplying in a rapid rate. By the end of the experiment a thick sediment covered the bottom of the bottles. Both strains had produced hemolysin on first isolation. This characteristic was maintained throughout the experiment. The only visible change took place in colonial morphology. On first isolation both strains were of the golden, large, flat colony type. After exposure to tricresol only small, moist, convex, cream colored colonies were produced. Elek (25b) described phenol-resistance of staphylococci and also mentioned resistance to quarternary amonium compounds. The latter are in general use for udder washing and for chemical sterilization of milking equipment. It may well be worthwhile to consider the resistance to disinfectants in the performance of milking sanitation.

II. Clinical Observations:

The following report includes observations made during the course of outbreaks of staphylococcal mastitis in two dairy herds in the vicinity of Edmonton, Alberta. The writer was consulted in both cases in agreement with the local veterinarians.

The two outbreaks are reported in a comparative form because of significant differences in their etiology, environmental factors which influenced them and in their response to control measures.

(1) Description of herds, facilities and management:

a.) Herd A is one of the largest dairy herds in this part of the province, having a size of two hundred Holstein cows. The milking part of the herd averages one hundred and thirty five animals. The herd is managed in loose housing, the premises consisting of a loafing barn, open wind-protected feeding area and one-way

milking parlour. The milking parlour is equipped with seven "Surge" milking units connected to a single pipe-line. The milk is collected and stored in a bulk tank. The herd is looked after by a man and wife with twenty years experience in hand milking and two helpers with no previous experience. The general health of the animals is good. Most of them are high-rated and of known breed. The farm is operated generously by the owner and provided with modern equipment.

b.) Herd B: This herd has a size of forty-five Holstein cows of good breed. Milking and housing facilities do not differ significantly from those of Herd A. Management and work is carried out by the two owners of the farm. They are of a consciencious and progressive type and have fifteen years experience in dairy farming in Canada.

(2) Mastitis situation and history at the beginning of these observations:

a.) Herd A: In August, September, 1959 the milking part of Herd A was sampled for bacteriological examination. The reason for the examination was a steady increase in mastitis accompanied by a rapid decrease in productivity and profitibility of the herd. At that time the expenses for antibiotic drugs used in treatment had reached monthly amounts of $500.00 to $800.00. The laboratory examination produced the following results:

Total samples examined: 508

Total of infected quarters: 323

Infection due to staphylococci: 138

Infection due to streptococci: 87

Combined staphylococcal, streptococcal infections: 98

The herd records showed that mastitis first had exceeded the

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

acceptable limit of 15 % in autumn, 1958. At that time the sudden spread, the appearance of strip cup samples and the fast response to antibiotic treatment were suggestive of streptococcal mastitis. Samples for bacteriological examination were not submitted. Treatment was performed by the personnel and consisted of udder infusion with penicillin and of intramuscular injection of the same antibiotic. The treatment succeeded in reducing the incidence of visibly infected quarters to a tolerable level. However, no effort was made to test the herd for the completeness of recovery or for the possible persistance of subacute infection. New cases were treated by udder infusion with penicillin as long as flakes in the milk persisted. In spring, 1959 a remarkable increase in mastitis was noted again despite penicillin treatment. A number of treated animals showed only a short temporary response or none at all. Therefore, treatment with penicillin was abandoned altogether in the herd and another antibiotic, terramycin, was used instead. For a period of about two months the results of the treatment seemed satisfactory. Then the response to the new antibiotic began to diminish. The number of visibly infected animals grew steadily. At the same time the milk production, even of apparently normal animals began to decrease. In August, September, 1959 the production was 35 to 40 percent below the average to be expected from a herd of this size and breed.

b.) <u>Herd B</u>: Prior to these investigations Herd B had a long record of excellent management and of low incidence of mastitis. Only limited seasonal outbreaks had occurred. Laboratory investigation showed that various species of streptococci were responsible for these. In fall, 1959 the

mastitis situation suddenly became serious. Within two to three weeks one-third of the animals showed infection in one or more quarters. Treatment seemed to have little or no effect. At this stage samples of all milking animals were taken and sent to the Provincial Dairy Laboratory for bacteriological examination. This examination gave the following results:

Total of samples: 144
Total of infected quarters: 45
Infection due to staphylococci: 39
Infection due to streptococci: 3
Combined staphylococcal streptococcal infection: 3

During an early appraisal of the situation with the owners of the herd, the following observations were made: the owners were very consciensciously trying to keep up with the latest progress in dairy management. They were steadily searching for improvements in milking facilities and hygiene, and kept several dairy journals as sources of information. This open-minded attitude has its reflections in the dairy farm which can in many ways be considered exemplary. On the other hand, the tendency to experiment has led to grave mistakes particularly in the use of antibiotics. The owners were under the impression that the use of these drugs, even without clinical necessity, would improve the general health of the herd. So they applied them generously in a prophylactic way, especially in spring and fall. During my first visit on the farm I found a big chest filled with a large variety of antibiotics. The selection of these products was guided by the advertisements in the dairy journals. The owners had no idea of the action and limitations of the individual drugs.

The treatment of mastitis cases was performed similarly to that in herd A: One dose per day as long as flakes persisted in the milk. No bacteriological examination was done after treatment.

(3) Mastitis control program:

After both farms had been thoroughly inspected with special attention to housing, equipment and hygiene, a program was worked out directed particularly towards the control of staphylococcal mastitis. Such a program had to be guided by knowledge of the ways of transmission, the mode of action and the characteristics of the organism. To eliminate experimenting and guessing as far as possible considerable basic instruction had to be given.

a.) Instructionof personnel: Human error and ignorance play a very important role in the mastitis problem. The knowledge of the dairy man simply does not keep pace withthe rapid development of equipment, sanitation, and therapy. The dairy industry readily accepted the modern methods of herd management without a clear understanding of the effects of these methods on the animal. Lack of knowledge is greatly responsible for the present mastitis problem and for the inability to bring the disease under control. The first step in a mastitis control program must, therefore, provide for adequate instruction of the persons concerned with the management of the herd. During these investigations considerable time and effort was spent in giving the personnel a basic idea of the character of the disease and of its agent The instructions included:

Predisposing factors, with particular emphasis on the damage caused by improperly adjusted milking machines.

The agents: In these cases, staphylococci. Size, habitat, rate of multiplication, ways of transmission and pathogenic potential were explained in simple terms.

Therapy: Purpose and action of various drugs was outlined roughly. It was made clear that the control of mastitis is mainly a matter of herd management and that chemotherap must be considered a last resort.

This theory was repeated on every occassion during this work until it finally became evident that its principles began to guide the work subconsciously.

b.) Elimination of predisposing factors by herd management: The controlof mastitis is mainly a problem of proper herd management. The first steps in the program were therefore directed towards the elimination of environmental factors predisposing the animals to infection.

i.) Inspection and improvement of outside areas: The areas around the housing and feeding facilities were thoroughly inspected.

Farm A: On their way to the housing barn the cattle had to cross a mud hole deep enough to bring the udders in direct contact with the mud. This hole had never dried up due to drainage of surplus moisture from the housing barn. During the whole warm season the cattle appeared for milking covered with a thick crust of dirt up to the abdomen. Proper udder hygiene was almost impossible under these circumstances. Similar conditions were found in the feeding area. This area surrounded by a solid wooden fence, did not have proper drainage. Thus rain and manure had converted the area into a sump in which the cattle wallowed several hours every day.

When these hazards were indicated, both areas were cleaned

out, filled up with gravel, and provided with drainage pipes. A hard surface will be provided for some areas around the housing barn this spring.

Farm B: No objectionable conditions were found in the outside area.

ii.) Inspection and improvements of housing: This area provides shelter for herds during the cold season. In such confined spaces the animals are completely dependent on the conditions maintained by the management. Four factors are of particular importance in providing healthy housing conditions: Elimination of draught, good ventilation, proper bedding and adequate irrigation.

Farm A: Ventilation openings under the roof of the barn had proved to be inadequate for the number of animals. Therefore, two doors on opposite sides of the barn were kept open regularly to allow escape of exessive heat and moisture. In this way a constant draught of cold air in the lower part of the barn created ideal conditions for chilling the udders. Closing of the doors and enlargment of the ventilation openings removed this hazard. The bedding was found to be excessively wet, saturated with faeces and urine, and too shallow. Animals lying down under these conditions would almost certainly suffer ill effects. The bedding was completeely removed and drainage facilities were installed. Then the ground was covered with a 15 inch layer of straw. In addition, enough straw was stored outside to ensure a replacement supply throughout the cold season.

Farm B: Bedding and irrigation were found to be satisfactory. However, again an existing draught condition throughout the barn may have contributed to lower the animals' resistance

against mastitis. The floor of the loafing barn was built on a slope from end to end. To provide for additional ventilation, two doors near the opposite ends were kept open, thus creating a constant draught throughout the length of the barn. Improvement was simple. The doors were closed and the ventilation was improved so that a sufficient air exchange could take place in a level close to the roof of the barn.

iii.) Supervision of milking equipment: There is ample evidence that machine milking results in a higher incidence of mastitis than does hand milking. It appears probable that machine milking causes irritation to the teats and the udder, since leucocyte counts and chloride countent tend to be higher in milk produced by this method. Therefore, great care should be taken to reduce this irritation of the gland to a minimum. This can only be done by maintaining the equipment at its highest efficiency. The vacuum, the inflations and the pulsators are the three main factors that will cause heavy damage to the udder when malfunctioning. The manufacturer of each machine recommends a definite vacuum at which it should be operated, and a definite number of cycles per minute at which the pulsator should be set. The teat cups and inflations are designed to work most efficientl under these conditions.

Farm A: Readings on a vacuum meter revealed that the machines worked under a vacuum far below the manufacturer's recommendations although the built-in gauge showed a correct reading. The gauge was replaced and an air leak in the line was found to be the reason for the loss of vacuum.

The inflations in use were of the wide-bore, synthetic rubber

type. They had been in operation for almost one year and had become rigid. Two of them had breaks. All inflations were replaced immediately by the narrow-bore, natural rubber type. This type has the advantage that it fits the teats more closely thus exerting a better massage action, reducing the penetration of outside air, and preventing the creeping-up of the teat cup during milking. The natural rubber material does not lose its softness. It may break sooner tha the synthetic material but it never becomes rigid. Storage of the rubber parts in lye between the milking times preserve the elasticity and extends the life time. On first inspection each pulsator was found to work at a different speed. The slowest was operating at 20 cycles and the fastest at over 100 cycles per minute. The munufacturer's recommendation is 45 cycles per minute. The moving parts of the mechanism were covered with sticky oil and dirt. All pulsators were taken apart and cleared in xylol. The moving parts were then lubricated with a thin film of fine oil. The speed was kept under strict control.

Farm B: Vacuum and pulsators were properly operating. The only improvement being necessary was the introduction of narrow-bore natural rubber inflations.

iv.) The milking procedure: It should always be realized that in machine milking the animal is exposed to a mechanical device and that the action of this device has to be controlle closely during the whole procedure. Even properly adjusted machines can do heavy damage to the udder if their action is not in accord with the natural process. The following milking procedures were introduced on both farms:

Stimulation of milk let-down: Any factor that will reduce

the time the machine is applied to the animal will help to prevent damage. The milk flow was stimulated by the udder washing procedure and by milking of several streams of fore-milk from each quarter by hand into a strip cup. When this stimulation was carried out properly, the milking time was reduced by about one-quarter.

Milking: The machine was attached within one minute of stimulation. The cow should then be milked out within four to five minutes.

Removal ofmachine: Teat cups were removed as soon as milking was complete because immediate damage to the udder tissues will result if the machine is left on too long. Directions were given that the cups should not be pulled off roughly and that the vacuum had to be released first.

Stripping: The quarters were stripped by hand. The hands of the milker were first soaked in disinfectant.

c.) Prevention of bacterial transmission: The bacterial agents of mastitis gain entrance to the udder via teat canal. This fact makes the prevention of transmission almost entirely a matter of careful udder hygiene. Of particular importance is such a hygiene in herds in which a high incidence of staphylococcal mastitis indicates the presence of virulent strains. The principal agent of streptococcal mastitis, S. agalactiae, has its exclusive habitat in the udder. It can not thrive outside the gland and is strictly an animal strain. Staphylococci, however, are widely distributed, have the ability to survive under unfavourable conditions and are parasites of man and animal alike, Udder hygiene may be neglected for a long time in well isolated herds without necessarily leading to out-breaks of streptococcal mastitis,

simply because of the absence of the agents. Staphylococci are continuously present in every herd and its environment. Any fluctuation in udder health may result in staphylococcal mastitis and any neglect of hygiene may lead to an epizootic outbreak of the disease. These important facts had been completely overlooked by the management of the two herds under observation:

Insufficient quantities and concentrations of disinfectants were used; contact of the udder with the disinfectant was far too short for bactericidal action; two or three pieces of damp cloth were used to clean and disinfect the teats of the whole herd; animals with acute mastitis were milked with the same teat cups that were used afterwards for healthy animals withou efficient disinfection; the milk of mastitic animals was fed to calves who possibly a little later sucked the teats of healthy cows or heifers. These were only a few of the observations made.

Udder hygiene is the vital step in any mastitic control program. Therefore, the following routine was introduced and supervised at every step:

Before milking every unit was provided with two large pails of disinfectant in the exact concentration according to the manufacturer's recommendation. In one of the pails face cloth were submerged in a number large enough to provide a separate cloth for each animal. The other pail contained a vessel for teat dipping. Each unit also was supplied with paper towels and a container with ointment for udder massage.

When the animals came in for milking only the healthy ones were admitted to the stalls. Cows with mastitic quarters were kept out of the milking parlour and milked after the

healthy part of the herd was finished. The infected animals had a red piece of wire attached to the neck chain to make them easily recognizable.

Sanitation routine began by soaking the udders thoroughly with disinfectant solution to soften up dirt crusts. Then a fresh face cloth from the disinfectant bucket was used to clean the teats completely. The cloth was folded and turned over after each quarter to decrease the change of transmission of possible pathogens from quarter to quarter.

The cloth was then disposed into a cardboard box. Now three to four streams of fore-milk from each quarter were milked into a strip cup and checked for the presence of flakes. Then the machine was put on. After milking and stripping, the teats were dipped into disinfectant using the dipping vessel and solution from the second pail. A paper towel was used to dry off the teats. Massage of the teats with small quantities of ointment concluded the procedure. Any animals showing external udder injuries were treated immediately.

Before the milking machine was put on another animal, the cups were submerged in disinfectant for at least 30 seconds. The efficiency of this procedure is demonstrated in table No. VII.

Exactly the same procedure was followed in milking the mastitic part of the herd.

However, heavily infected quarters were milked out by hand. Special care was taken not to spill any secretion on the floor. The soiled paper towels were thrown into a separate cardboard box and burnt immediately after milking. Mastitic milk was poured into a drainage hole and washed down with large portions of water. During the entire milking period

Table No. VII

Effect of Udder Washing on the Number of Staphylococci.

Number of organisms per $inch^2$ of skin of teats.

	Average Count
No. examined	10 each time
Before washing	9 200
After washing	900
After milking	1 200
After dipping and drying	1 050

Samples were taken in Herd A during the period of high incidence of staphylococcal mastitis. Only teats free of manure and dirt crusts were examined.

For sampling sterile cotton swabs soaked in peptone water were used. The individual swabs were then placed into test tubes containing 5cc amounts of peptone water. After agitation of 5 min. amounts of 0.1 cc were streaked on the surface of oxblood agar plates. Plates were incubated for 24 to 48 hours at 37°C.

the personnel was advised to soak the hands in disinfectant as often as possible, but definitely after handling of mastitic animals, and to use paper towels for drying.

The face cloths were boiled in a detergent for an hour after each milking period and then kept submerged in a pail of disinfectant until the next period.

The milking parlour was flushed out with water and scrubbed with a 1 oz/gal/solution of "Roccal" between the milking periods.

d.) Treatment of infected animals: A bulletin of the Ontario Veterinary College (7) begins the chapter on the treatment of mastitis with this statement: " Probably for no other disease of animals has treatment been so widely used and abused as for mastitis." Numerous personal observations confirmed this statement. The "shot gun" therapy practiced in many herds by putting at random large amounts of various drugs into the animals may temperarily check an infection but will never result in a clean herd. Chemotherapy should never be left to empirical trial. It should be carefully planned and - particularly in staphylococcal mastitis - should be accompanied by adequate improvements in management. In the two herds under observation, the writer followed this procedure:

i.) Check into mastitis and treatment history: The knowledge of previous outbreaks, of drugs used and of the results of treatment is of great value in the planning of efficient treatment. In both herds large amounts of penicillin and terramycin, and in herd B streptomycin in addition, had been used with decreasing response. Therefore, it was decided to exclude these drugs from further use although

several strains of staphylococci, isolated from the herds as pathogens, still showed in vitro sensitivity. This measure was considered desirable because of the urgency of effective treatment. It was based on the knowledge that at least some of the strains had been exposed to one or more of the drugs and on the assumption that they might have developed some degree of in vivo resistance.

ii.) Choice of drugs: There is a great number of products on the market for the treatment of staphylococcal mastitis. It is of importance to find the best one for a specific case. The drug should have high inhibitory qualities and should have a spectrum covering all strains isolated as pathogens. Several methods of determining in vitro sensitivity to various drugs were investigated. The following method was found most reliable:

Serial dilutions of the investigated drug were made in 0.8 % saline. A 1 ml amount of each dilution was added to 9 ml melted nutrient agar and mixed thoroughly. Then the mixture was poured into a sterile Petri dish. Each plate was subdivided externally with a grease pencil in as many segments as there were strains to be tested. The corresponding medium segments were inoculated with the strains isolated as agents and the plate was incubated for 18 hours at 37° C.

In this way any drug that was not fully effective in low concentrations against all the strains was excluded.

For both herds "Neothion", a product incorporating neomycin and thiostrepton, was found to be most effective. The advantage of a combination of two antibiotics in one drug will be discussed in a later part. The manufacturer of "Neothion" claims that it has synergistic action when used against staphy-

lococci.

For alternative or combined therapy a second drug with a different mode of action was tested and in some stubborn cases, sudcessfully applied. The choice was "Hibitane." Hibitane is a fairly new, liquid, organic disinfectant developed in England. It is bactericidal in strong concentrations and highly bacteriostatic in weak concentrations. Even in strong concentrations it causes little irritation of the tissues, and it is very stable in the presence of organic matter. Infusions of undiluted Hibitane in doses of one-half ounce per quarter per day proved very effective in advanced cases.

iii.) Method and dose of udder infusion: Personal observations suggested that in many cases treatment of staphylococcal mastitis failed because of poor administration procedure and of miscalculation of dosage. Procedure and dosage should be guided by the time-concentration factor, i.e. a particular drug is only effective if it can act at a sufficiently high concentration for a sufficient length of time. Drugs for udder infusion ar sold in one-dose lots, i.e. one lot contains enough units of the drug to give an effective initial concentration. The further dosage must be calculated in a way that ensures the maintainance of an effective concentration throughout the period of treatment. In the two herds, the following dosage was tried successfully and without ill side effects:

First treatment: 1½ to 2 doses depending on size of quarter.

After twelve hours: 1 dose

After twelve hours: 1 dose

After twelve hours: $1\frac{1}{2}$ to 2 doses

The reasons for the variations in dosage were: A high initial dose ensures, even in remote parts of the gland, an effective concentration thus inhibiting the emergence of resistant mutants. The next two single doses make up for the gradual decrease of concentration over a period of twelve hours. A high final dose is directed against cells that may have survived treatment in remote parts of the udder and may have acquired some resistance. In my opinion it is important that once treatment of staphylococcal mastitis is started, never less than four doses per quarter should be administered. Anything less than this minimum will almost certainly result in the survival of some staphylococci and probably in the emergence of resistant mutants. In these two herds six doses were considered the maximum for one course. Continuation beyond this limit seemed to result in toxic side effects, irritation of the tissues and drying off of the quarter. In cases that showed no satisfactory response after six doses a resting period of six days was allowed and then a second course with an alternative drug administered. It has been said above that each dose contains enough units to give an effective concentration in the quarter. This is only true if the whole content of the tube or syringe gets to the site of the infection. Manifestly there was such an obvious neglect of this fact that an exact procedural scheme was drawn up:

"Clean the orifice of the teat with alcohol. Dip the nozzle of the tube or syringe (warm up to body temperature before administration) into vaseline to avoid

injury to the lining tissue. Insert nozzle carefully. Hold end of teat between two fingers of left hand. Squeeze complete content of tube into teat. Pull out tube and close off teat orifice by pressure between two fingers. Streak with the other hand several times upwards along the teat to force most of the drug up into the quarter. Now squeeze off upper end of teat with two fingers and exert a circulary massage action upwards against the quarter. Still holding the teat closed massage with the other hand from the teat on upwards all around the quarter."

By following this procedure no significant loss of dosage can occur and the drug is distributed throughout the quarter without loss of time.

iv.) Prophylactic treatment: The prophylactic use of antibiotics has become a frequent practice in dairying. One or two doses per quarter are believed to prevent infection. On some occasions the writer has observed that animals were treated with small doses shortly before drying off. Such a practice is not only a senseless waste of money but may also lead to the ill effects mentioned before. Antibiotics in mastitis control have to be reserved for specific treatment. Any misuse may decrease the chances of success of specific treatment.

In staphylococcal mastitis there is only one preventive treatment offering some protection: specific immunization, Tentation trial of a vaccine prapared from five phage types of staphylococci isolated from acute mastitis gave favourable results:

v.) Retesting after treatment: No treatment should be

considered successful unless confirmed by a bacteriological examination three weeks after the last dose. Milk from infected quarters will in most cases appear normal after two or three days of treatment, but in staphylococcal mastitis, as opposed to streptococcal mastitis, this must not lead to the conclusion that the quarter is normal. In the two herds investigated, ten percent of the treated quarters were still mastitic three weeks after treatment, although in most of them all clinical signs had disappeared. The frequent omission of bacteriological re-testing may be one of the reasons for the difficulties encountered in the control of staphylococcal mastitis.

c.) Other measures: In this paragraph several other measures employed in the control program in the two herds may be stressed briefly, as they are of a more general nature and do not particularly apply to staphylococcal mastitis:

i.) All animals to be dried off should be examined for mastitis and treated if necessary.

ii.) All new additions to a herd should be bacteriologically tested before contact with the herd is allowed.

iii.) All animals suffering from frequent recurrence of acute mastitis and showing little response to repreated treatment should be eliminated from the herd.

iv.) Animals under treatment for mastitis should be kept on a reduced diet of roughage. No rich food concentrates should be fed. A moderately producing gland responds better to treatment.

v.) The use of the California Mastitis Test (28) as a field test for the early detection of mastitis is highly recommended. The test is very sensitive, easy to read and

requires only about thirty seconds per animal. The test enables the herd management to detect the presence of mastitis long before clinical symptoms appear.

vi.) Of great value in the control of mastitis is a herd record book. Such a book should contain the complete history of each animal including mastitis incidence and kind of treatment.

(4) Course and results of the control program:

a.) Herd A: From the evidence presented it is obvious that the high incidence of staphylococcal mastitis in this herd resulted from a number of mistakes in management as well as in treatment. When this investigation began it was difficult to decide whether the staphylococcal outbreak was due mainly to generally poor health resulting from poor management or to a selection of virulent and resistant strains by inadequate treatment methods. The coexistance of a high incidence of streptococcal infection indicates that the conditions in the herd were favourable for mastitis in general. The fact that two of the three types of staphylococci isolated as agents showed *in vitro* resistance to the two antibiotics (terramycin and penicillin) used for almost a year in the herd suggests that emergence and selection of resistant mutants may have taken place. The situation, as it was, did not allow much time for experimentation. The program which was put into effect immediately was developed under three headings:

Rapid improvement in management;

Blocking, as far as possible, of all ways of further spread;

Choice of the most effective treatment and continuation

of treatment until satisfactory laboratory results were achieved in each individual quarter.

Much stess was laid on the necessity of following accurately every single step of the program. It seems to be of particular importance in the control of staphylococcal mastitis that every measure taken is carried out and maintained with the greatest care. The organisms exhibit such a stubborn persistence that they can be controlled only by most pedantic methods.

Five weeks after the program was put into effect the herd was re-tested and the following results obtained:

Total of samples examined: 512
Total of infected quarters: 140
Infection due to staphylococci: 68
Infection due to streptococci: 20
Combined staphylococcus, streptococcus infection: 52

In these figures are included the new cases which appeared in the five weeks between the first and the second testing. After another five weeks a third laboratory test was performed:

Total samples examined: 504
Total of infected quarters: 42
Infection due to staphylococci: 32
Infection due to streptococci: 4
Combined infection: 6

Very few new infections occurred during this period. The third test was performed in December, 1959. The incidence expressed in the results of this test correspond with the present situation. There is no actual mastitis problem existing in the herd now. Response to chemotherapy can be

summarized: The large majority of streptococcal infections could be cleared up in the first course of antibiotic treatment. About forty percent of the staphylococcal infections required either prolonged treatment or a second course of treatment in which Hibitane was used as an alternative drug. Two animals did not respond to any treatment (including several other antibiotics) and were eliminated from the herd.

Herd B: Contrary to Herd A there were very few adjustments of management necessary. The herd was kept under very clean and healthy conditions. It had only a few minor mastitis outbreaks prior to the time of observation. The fact that there were only two streptococcal infections in the herd at the time of the first examination suggested that the animals had good resistance to infection and little predisposition to mastitis. Yet suddenly a considerable percentage of quarters contracted staphylococcal mastitis. Explanation can only be hypothetical but the following seems feasible: The owners of this herd did much experimenting with new drugs. They used preventive doses in fall and spring and on drying animals. So the common udder flora was frequently upset. The more sensitive species were eliminated and some resistant ones survived. The species exhibiting the greatest ability to vary adaptively would survive in such a selective process. It has been shown that staphylococci possess this ability to a considerable extent. In fact among the normal udder flora there is no other organism that can compare in survival ability with the staphylococcus. So, by the misuse of drugs, normally competitive organisms were destroyed. The variety of drugs

used may also have resulted in a selective process among the strains of staphylococci. Strains with the greatest ability to mutate may have been the final survivors. This is suggested by the results of treatment given below. Such strains, however, may have "colonized" the herd widely but, for a considerable period, may have established a peaceful co-existence. Suddenly a large part of the herd may have been exposed to some environmental factor weakening the general resistance of the animals. This may have been a chill, sudden climatic changes, an abrupt change in diet, or some other factor. The temporary weakening of resistance may have been enough to offer an opportunity to the pathogenic potential of the staphylococci, thus precipitating a major outbreak of mastitis.

The course of the control program, also was quite different to that in Herd A: Only two new infections occurred during the whole period suggesting that the general health of the animals was again good and that any factor predisposing to mastitis was temporary in nature. On the other hand, the response to treatment was not nearly as good as in Herd A. In most cases, six doses of Neothion per quarter and the additional administration of Hibitane were necessary for complete cure. Five of the quarters did not respond at all. A large variety of drugs were tried without success over a period of several months. Then two of the quarters cleared up after treatment with erythromycin. A re-test

performed two months after treatment was concluded, showed that fifty percent of the treated quarters still harbour staphylococci in moderate numbers associated with a very slightly raised leucocyte count in milk.

DISCUSSION

1. Revision of conventional ideas on bovine mastitis:

(1) Changes in herd management:

The general idea of this work was to demonstrate that in the Province of Alberta bovine mastitis in general and staphylococcal mastitis in particular has become a problem that can not be controlled any longer by the conventional approach to this disease. In a short period of time fundamental changes have taken place in the dairy industry. Milking, housing, feeding, breeding, and sanitation have been mechanized or stream-lined in the interest of higher production and greater returns. The old time farm has changed into a modern factory, yet the animal around which this modern industry was built has remained unchanged. It must be realized that modern methods of dairying have put a heavy stress on the health and physiology of the animal, especially on the milk producing gland. It is obvious that an organ continuously exposed to such a stress will possess a decreased resistance to infections diseases. Thus mastitis, of little importance in the days of hand milking, now has become a wide spread and costly disease. When chemotherapy was introduced it seemed to offer a solution to the mastitis problem, too easy a solution, it seems now. Misconception of the mode of action and of the limitations of these drugs has greatly decreased their beneficial effect and has in some respects contributed to the complexity of the mastitis problem.

(2) Changes in the etiology of bovine mastitis:

Modern ways of herd management have influenced not only the importance but also the etiology of mastitis. *S. agalactiae*

used to be the classic mastitis agent, which was responsible for eighty percent of epidemic outbreaks of the disease. This organism is metabolically strictly dependent upon the mammary gland. No other permanent reservoirs are known. Once eliminated from a herd, it could only be reintroduced by direct contact with infected foreign animals or by some intermediate vehicle. Whenever *S. agalactiae* appears in a herd it will cause disease to some degree. It never becomes established as part of the normal udder flora. With the arrival of chemotherapy in the treatment of mastitis the rôle of *S. agalactiae* as the leading agent was rapidly ended. This organisms was highly sensitive to chemotherapy and has retained this sensitivity unchanged to all antibiotics except streptomycin. Its restricted habitat and the stability of its characters made this organisms an easy target for chemotherapy resulting in a rapid decrease in its importance as a mastitis agent.

The position of chief importance in the etiology of mastitis is now gradually being taken over by staphylococci. These organisms exhibit many characteristics opposite to those of *S. agalactiae*: They have many reservoirs in nature; they are only facultative pathogens; they belong to the normal udder flora; they exhibit a great capacity for variation and an ability to develop resistance to chemotherapy. The pathogenicity of staphylococci has been increased only relatively by a general decrease of udder health. The stress of over-production results in lowering of resistance to such a degree that even staphylococci of little virulence may cause disease. Therefore, when assessing the pathogenic

potential of staphylococci in connection with mastitis, one should always bear in mind that the site of invasion is continuously in an abnormally low state of resistance.

11. Some considerations of particular interest in the etiology and treatment of staphylococcal mastitis:

(1) Significance of the "normal udder flora"

In the writers opinion the bacteriological situation in the udder resembles very closely that of the human nose cavity and throat. Here the common population is similar or identical to that of the udder. We also know that it is impossible to destroy this flora permantly or to protect the individual from the infection. Despite that, however, human medicine does not recognize this population as "normal flora," living in a stable, harmless relationship to the host. Here we are conscious of the fact that a number of the common inhabitants are potential pathogens and that chemical, thermal or physiological changes in their environment may also change their peaceful attitude towards the host. We further know that there is no stable balance amongst the microbial population. The co-existence of the various kinds is rather competitive than symbiotic of nature. Fluctuations even within the same species are possible at any time. More virulent strains may supplant the less aggressive ones. Strains which have adapted themselves to certain drugs may take the place of relatively sensitive ones. Unnoticed by the host, the action of one kind on the environment may pave the road for the invasion by another more powerful pathogenic agent. So, with respect to the bacterial population of the human nose and throat, we may accept the following facts:

a.) The two loci in the human body commonly harbour potential pathogens.

b.) There is never a premanent balance in the host-parasite relationship, safeguarding the host against changes in composition and virulence of the bacterial population.

c.) The presence of this population therefore, constitutes a steady source of danger to the host.

d.) While it is not possible to eliminate this population permantly from the two loci, it would be ignorant to belittle its potentially pathogenic character by terms like "normal flora."

The writer consciously has placed emphasis on the foregoing paragraph because he blames the limited success in controlling bovine mastitis to a certain extent on the ignorance toward the reservoirs of potential agents within the udder. He also considered the mentioned parallel in human bacteriology as a possible lead in the search for reasons explaining the recent increase in staphylococcal mastitis. It has been said that in the human nose or throat, thermal, chemical or physiological changes may bring about the activation of the pathogenic properties of part of the bacterial population. Evidentally the same holds true with respect to the cows udder. This organ is exposed to such changes to an even higher degree than the two loci in the human body. The location as an appendix of the body leaves it much more unprotected against thermal changes and external injuries. During the lactation period the udder is constantly placed under a heavy stress, thus offering diminished resistance to bacterial infection.

(2) "Mastitis staphylococci"

As mentioned before the eighty strains under investigation in this work were isolated as agents from well established cases of acute mastitis and were associated with a leucocyte count of at least 1,000,000 per ml. From the clinical and bacteriological evidence it must be concluded that all eighty strains, independent of their other differences, have three properties in common; Pathogenicity, infectivity and virulence. It was not possible to associate these three properties specifically with any of the other characters. It seems, therefore, not justifyable, as it is frequently done, to speak of "mastitis staphylococci" as a well differentiated group including only hemolytic, mannite-and coagulase positive strains. Evidence suggests that any strains of staphylococcus may under certain circumstances assume the properties necessary to cause disease. An extreme example in this respect is presented by strain No. 60. It was non-hemolytic, did not ferment mannite and did not produce coagulase, yet repeated cultural and microscopical evidence identified the strain as agent in a severe case of mastitis. Cases like this may be rare but nevertheless they demonstrate the necessity for an imaginative approach to the work with staphylococci.

No absolute correlation could be established between the various characteristics as exhibited in vitro by the eighty strains investigated. A very high coincidence seems to exist between mannite fermentation and coagulase production.

If the observations on these eighty strains are considered representative for mastitis staphylococci in general, then the following statement may be permissable: Staphylococci, isolated from the bovine mammary gland may for unknown

reasons exhibit large phenotypic or genotypic differences *in vitro* and yet their pathogenic potential may remain unchanged. The diagnosis of staphylococcal mastitis can therefore be based reliably only on clinical and microscopical evidence. The characters exhibited *in vitro* by individual strains of staphylococci isolated from suspected disease processes are of secondary significance.

(3) *Chemotherapy and the emergence of drug resistant strains:*
The emergence of drug resistant strains due to therapeutic failure is one of the greatest hazards in the chemotherapy of staphylococcal mastitis. The first step towards a limitation of this hazard should be to enforce by law the restriction of the use of antibiotics to competent persons. It has become common practice on dairy farms to hold a supply of antibiotics on hand and to inject a syringe full or two as soon as some flakes in the milk shows the presence of acut mastitis. In many instances this dose is sufficient to suppress visible mannifestations of the disease for a certain time but it does not preclude the possibility of persistance of the disease in a subacute form. If the theory is true that drug resistance frequently develops in several steps by survival of the most resistant individual cells in successive generations than this process can only be blocked by initiating and maintaining drug concentrations high enough to inhibit the first-stage resistant mutants. However, this procedure offers no protection against one-step mutants to a high level of resistance. This phenomenon has been observed by the writer in two instances during treatment with streptomycin. In these two cases the initial treatment was highly successful but within

a few days relapse occurred and the drug proved then completely ineffective. *In vitro* sensitivity tests showed that the strains had become resistant to concentrations beyond the level practicable in treatment. The best way to prevent the formation of resistant mutants, therefore, appears to be combined therapy. This solution which was suggested by Ehrlich (29b) has a rationale based on genetic principles: "If one cell in 10^6 mutates to resistance to one drug, and one in 10^6 to another, only one in 10^{12} will develop both mutations simultaneously." However, in combined therapy the selection of the components has to be based on a clear understanding of their mode of action, e.g. A combination of penicillin and a bacteriostatic agent could diminish the effectiveness of penicillin considerably. The bacteriostatic agent reduces the metablic activity of the organism. Penicillin on the other hand, can only exert its lethal effect on organisms in full metabolic activity. Thus the selectinn of the components of combined therapy has to be directed toward an additive mode of action. Several prefabricated mixtures for the treatment of staphylococcal mastitis are now on the market. Some of these products claim synergistic action, e.e. greater than additive action. The writer has observed this phenomenon on several occasions with neomycin and streptomycin. *In vitro* tests showed that some strains, exhibiting low sensitivity to these drugs when used individually, were completely inhibited by a mixture of the two.

From the evidence presented here it becomes obvious that drug treatment of staphylococcal mastitis should not be subject to "hit or miss" experiments by the layman. Also

it should never become an alternative to proper barn and milking hygiene. Too many dairy men already rely upon the use of the so-called "wonder drugs" as a time-saving and fool-proof device to control mastitis. Drug treatment should always be considered a last resort and should therefore play only a subordinate role in the control of mastitis in properly managed herds.

CONCLUSION

From the evidence presented in the early portion of this work on staphylococci as agents of bovine mastitis it may be concluded that:

1. These organisms exhibit great variability in colonial morphology and pigmentation. None of these characters can be considered reliable for identification and classification.
2. There appears to be no regular correlation between any of the characteristics of mastitis staphylococci studied in vitro. None of these characteristics can be depended on to indicate the pathogenicity of the organisms in vivo.
3. The laboratory diagnosis of staphylococcal mastitis should be based on the evidence presented in each individual sample examined. Any pre-existing ideas about the characteristics of so-called pathogenic staphylococci should be abandoned.
4. Susceptibility of an individual staphylococcal strain to a pattern of phages seems to be highly persistant in vivo as well as in vitro. Phage typing, therefore, is a reliable aid to epidemiological investigation.
5. The number of antibiotic resistant strains of staphylococci recovered from milk and from infected cattle has significantly increased in recent years. Misuse of antibiotics is probably mainly responsible.

The later clinical observations were made during an apparently successful attempt to control staphylococcal mastitis in two Alberta dairy herds. The ideas guiding the adopted program may be expressed as follows:

1. Staphylococci are continuously present in every dairy herd. Factors lowering the resistance of the animals may at any time potentiate the pathogenic properties of staphylococci

and lead to disease.

2. Poor management, inadequate sanitation and ignorance about the etiology of staphylococcal mastitis are largly responsible for the increase in its incidence.
3. Empirically used antibiotics alone will never eliminate staphylococcal mastitis from a herd. The control program should include instruction of personnel, elimination of predisposing factors, prevention of transmission, careful sanitation and hygiene and scientifically selected treatment.
4. Chemotherapy should be considered a last resort in the control of staphylococcal mastitis. It should never be used for preventive purposes.
5. Chemotherapy should be used in the most effective way, ie. The various drugs should be selected on the basis of *in vitro* tests to determine their specific antibacterial activity calculated adequately and treatment should be continued till bacteriological examination confirms complete success.
6. Staphylococcal mastitis can be controlled only if every measure is carried out with the greatest accuracy and persistance.

Evidence presented in this thesis shows that staphylococci are rapidly becoming of chief importance as causal agents of mastitis in the dairy herds of this Province. There are also indications that the general incidence of mastitis is increasing. In a disease with the staphylococcus as one of its chief agents a "wait and see" attitude is inexcusable. Unless more vigorous and enlightened measures for the control of the disease are taken soon, the dairy industry in Alberta will suffer increasingly heavy losses in the near future.

References

(1) Hoard's "Dairyman", Editorial, Issues March-April 1959.

(2) Plastridge,N.W., J.of Dairy Sc.,Sept.1958,Vol.41,p.1141

(3) Alberta Gov.Dept.of Agric., Pamphlet April 1959.

(4a) Little,R.B. and Plastridge,N.W., "Bovine Mastitis", McGraw-Hill Book Comp.,New York,1946, pp. 63-97

(4b) ibid. p.167

(4c) ibid. p.190

(4d) ibid. p.191

(4e) ibid. pp.195-196

(4f) ibid. p.112

(4g) ibid. pp.192-193

(4h) ibid. p.423

(5) Merchant,I.A. and Packer,R.A., "Etiology, Diagnosis and Contro of Infectious Bovine Mastitis", Iowa State College,1944,p.14

(6) Lister,J., J.Path.Soc.(London),29:425,1878.

(7) Newbould,F.S. and Barnum,D.A., "Bovine Mastitis", Ont.Vet.Coll Bull.525,Nov.1957,p.8,9,34.

(8) Gorini,C.: 1902, cited by Evans, J.Inf.Dis. 18,437,1916

(9) von Freudenreich,cited by Evans, J.Inf. Dis. 18,437,1916

(10) Evans,A.C., J.Inf.Dis. 18,437,1916

(11) Lucet,R. ,Rec.Med.Vet.,6,423. 1889

(12) Minett,F.C. J.Path.Bact.,42,247, 1936

(13) Guillebeau,A.,cited by Jones, F.S.,J.Exp.Med.28,721,1918

(14) Steiger,P.,cited by Jones,F.S., J.Exp.Med.,28,721,1918

(15) Savage,W.G.,1907-1908,37.Ann.Rep.Loc.Gov.Board,London,App.B,4

(16) Jones,F.S., J.Exp.Med.,28,721,1918

(17) Carpenter,C.M.,J.Am.Vet.Med.Ass.,N.S. 20,317,1925

(18) Rolle,M.,Deut.Tierärztl.Wchschr.,40,257,1932

(19) Spencer,G.R. and Lasmanis,J., Am.J.Vet.Res.,XIII 49,500,1952

(20) Elek,S.D. and Levy,E., J.Path.Bact. 62,541-554, 1950

(21) Bryce,L.M. and Rountree,P.M., J.Path.Bact.,43,173-189,1936

(22) Williams,R.E.,and Harper,G.J., J.Path.Bact. 59,69-78, 1947

(23) Marks,J. and Vaughan,A.C.T., J.Path.Bact. 62,597-615, 1950

(24) Hale,J.H. and Smith,W., Brit.J.Exp.Path. 26,209-216,1945

(25a) Elek,S.D., "Staphylococcus Pyogenes and its relation to disease", E.& S. Livingstone Ltd.,Edinburgh and London, 1959, pp.114-151.

(25b) ibid. pp. 226 - 248

(26) ibid. , pp. 446 -447

Demerec,M., Proc.nat.Acad.Sc.(Wash) 31,16-24, 1945

Demerec,M., Ann.Mo.bot.Gdn. 32,131-138, 1945

(27) ibid. p.449

Barber,M., J.gen. Microbiol. 8,111-115, 1953

(28) Schalm,O.W., Bull. School of Vet.Med. U.of Calif, 1956

(29a) Dubos,R.J., "Bacterial and Mycotic Infections of Man", J.B.Lippincott Comp.,Philadelphia, 1958, p.107

(29b) ibid. p.686

(30) Hammer,B.W. and Babel,F.J., "Dairy Bacteriology", J.Wiley & Sons,New York, 1957, pp.329,213,

(31) Tanner,F.W., "The Microbiology of Foods", Garrard Press, Champaign,Illinois, 1946,

(32) Wilson,G.S. and Miles,A.A., "Topley and Wilson's Principle of Bacteriology and Immunity",Edward Arnold & Co.,London, 1948, p.611

(33) Mann,P.H., Can.J.Publ.Health,Vol.51,No.4,Apr.1960,pp 153/6